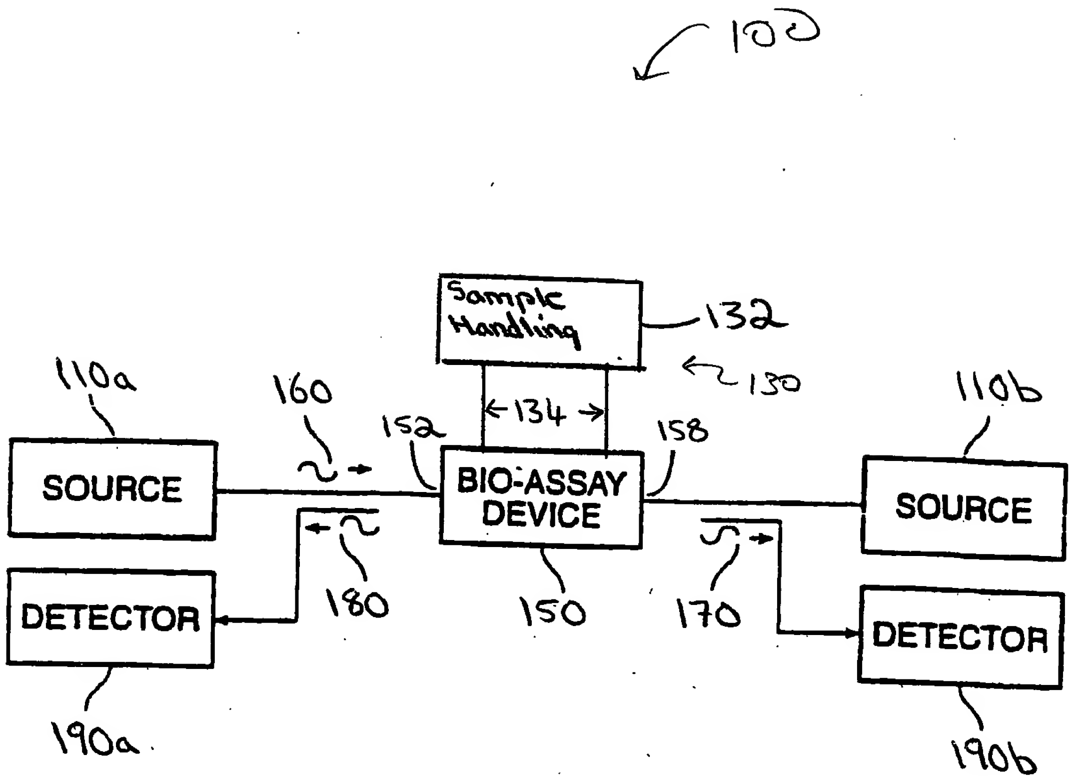
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Sample Handling
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130
134
110a
160
152
158
110b
SOURCE
BIO-ASSAY DEVICE
SOURCE
DETECTOR
180
150
170
DETECTOR
190a
190b

Fig 1

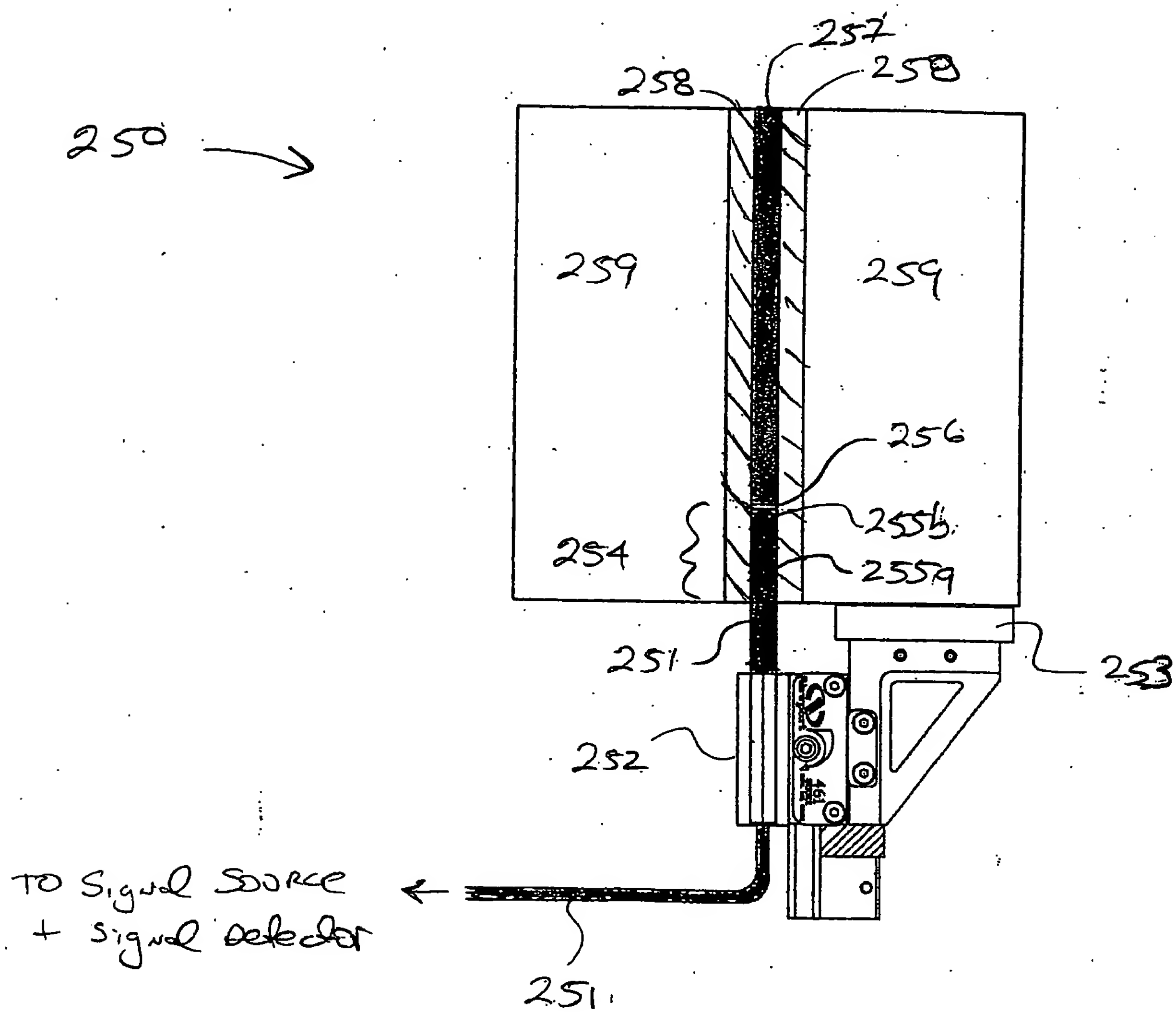


Fig. 2

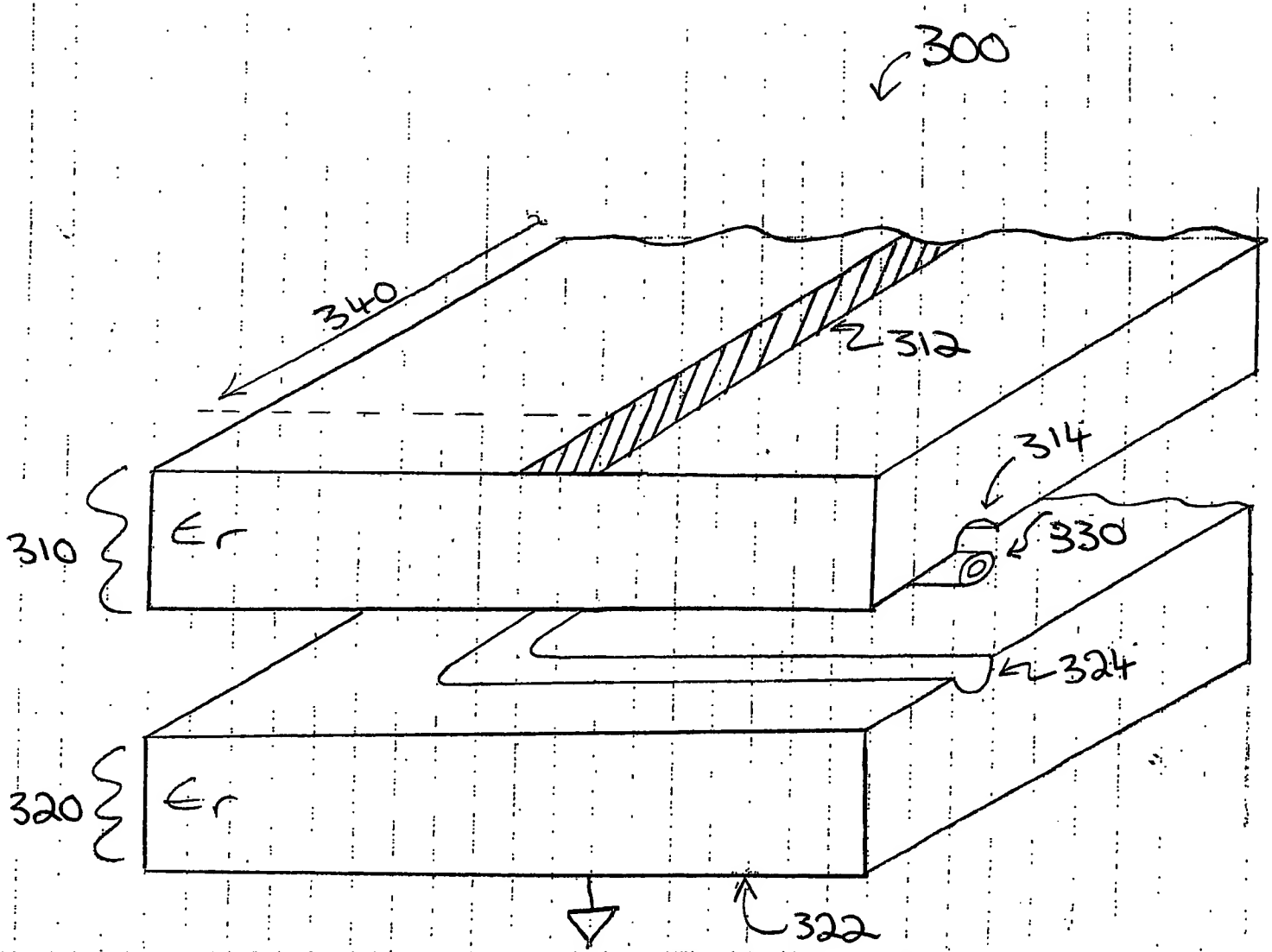
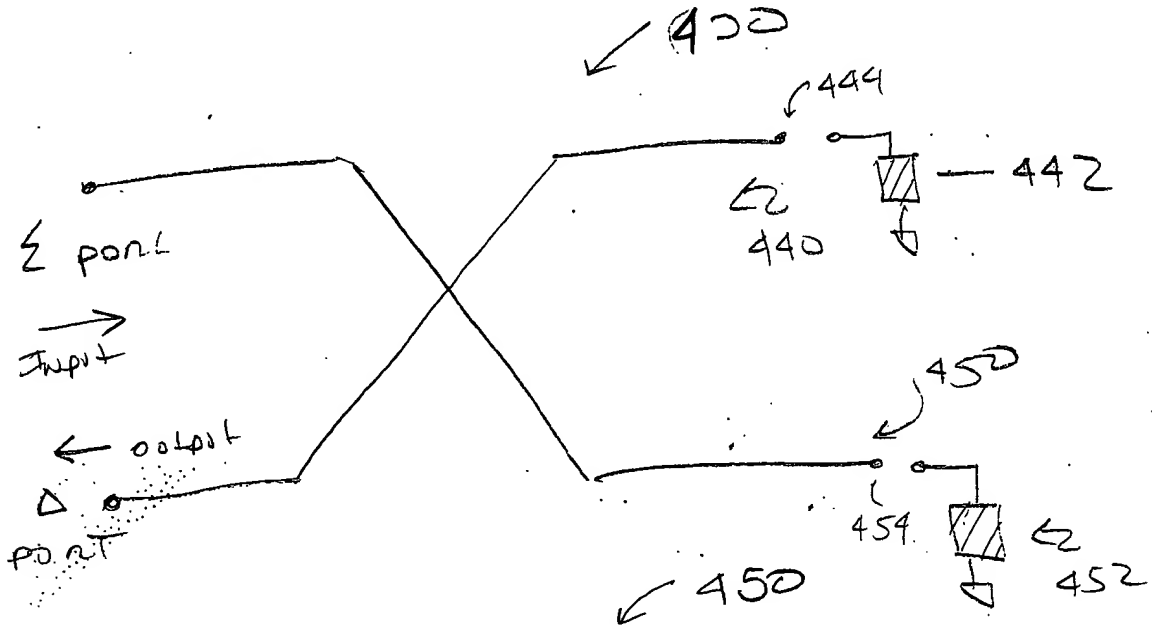


Fig. 3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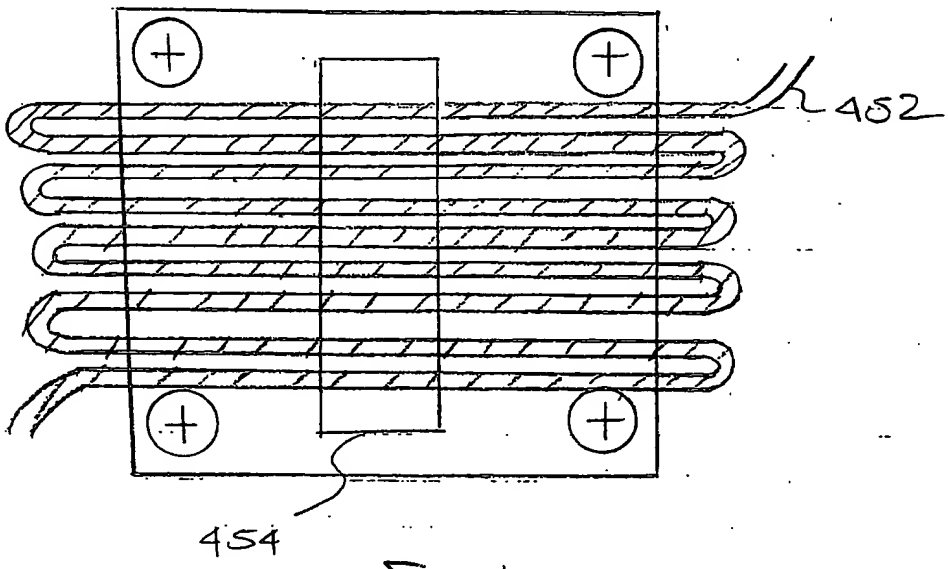
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454

Fig 4A

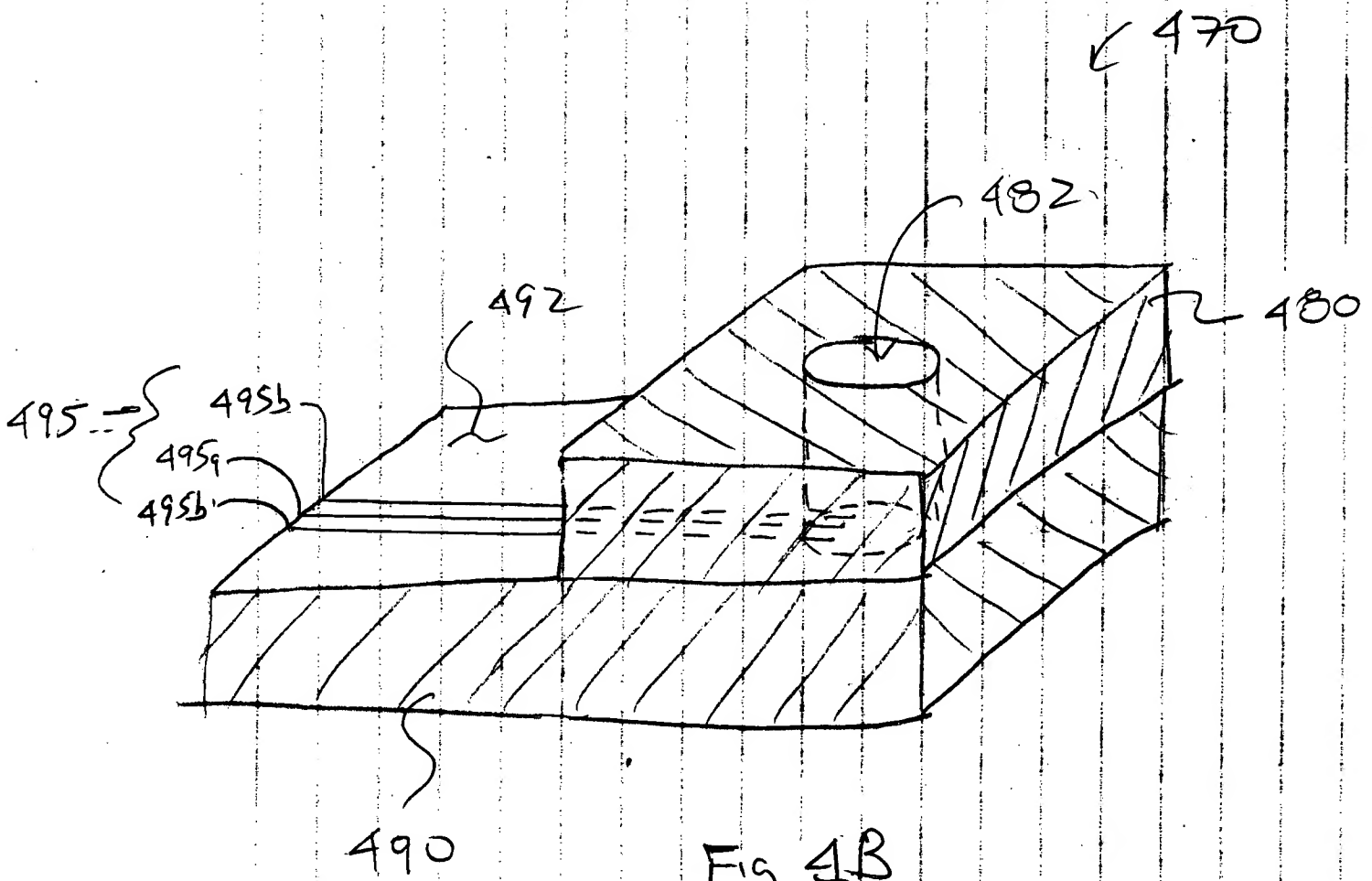


Fig 4B

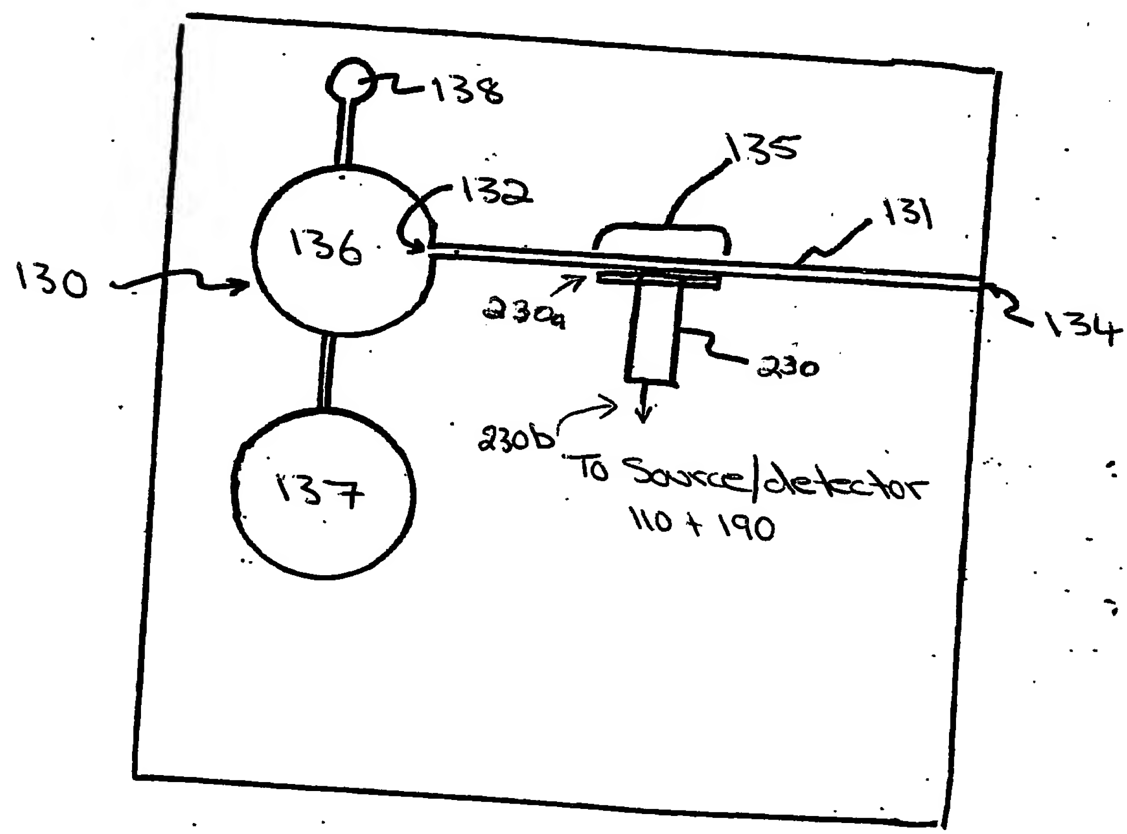


Fig 5

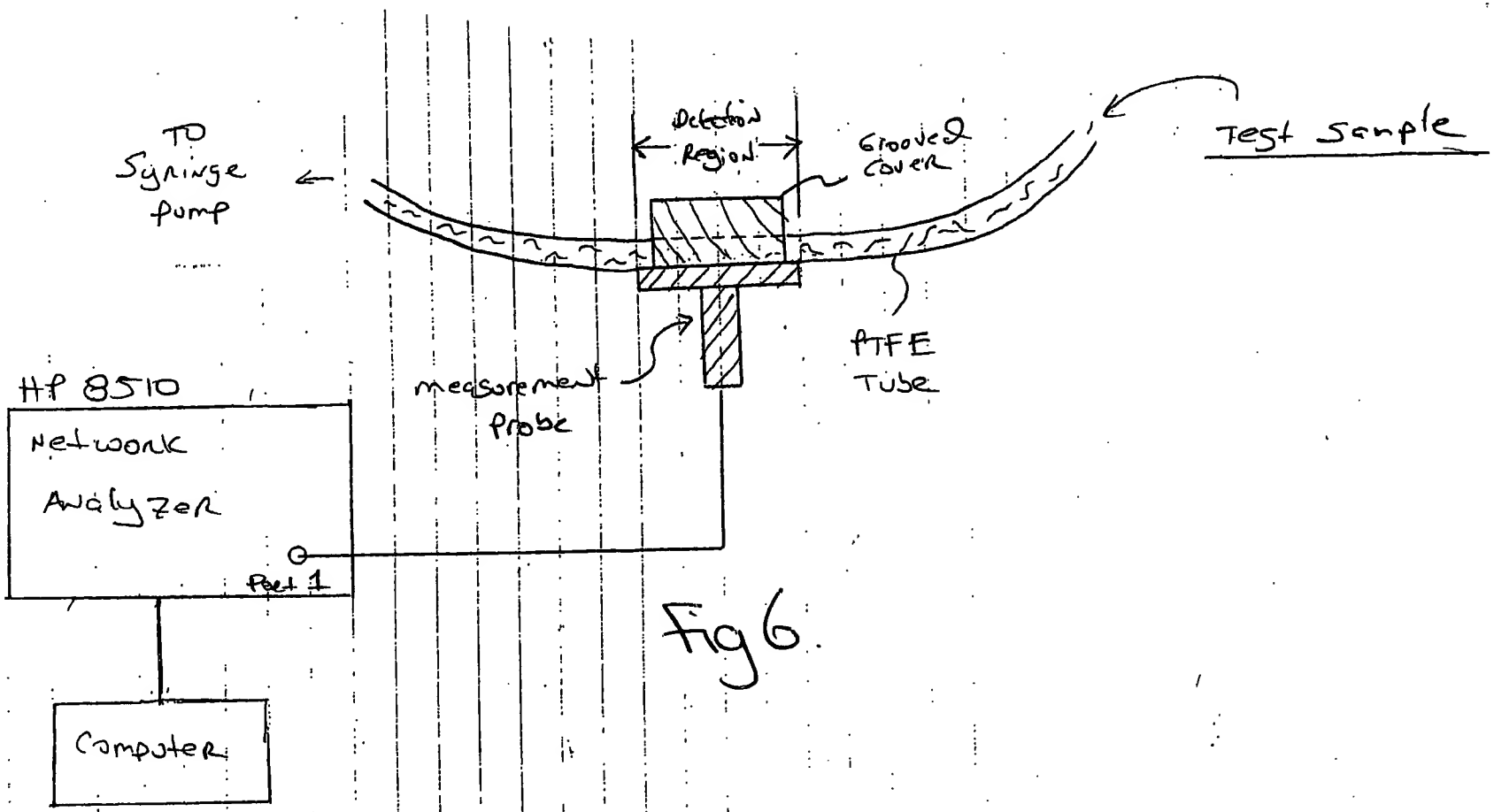


Fig 6.

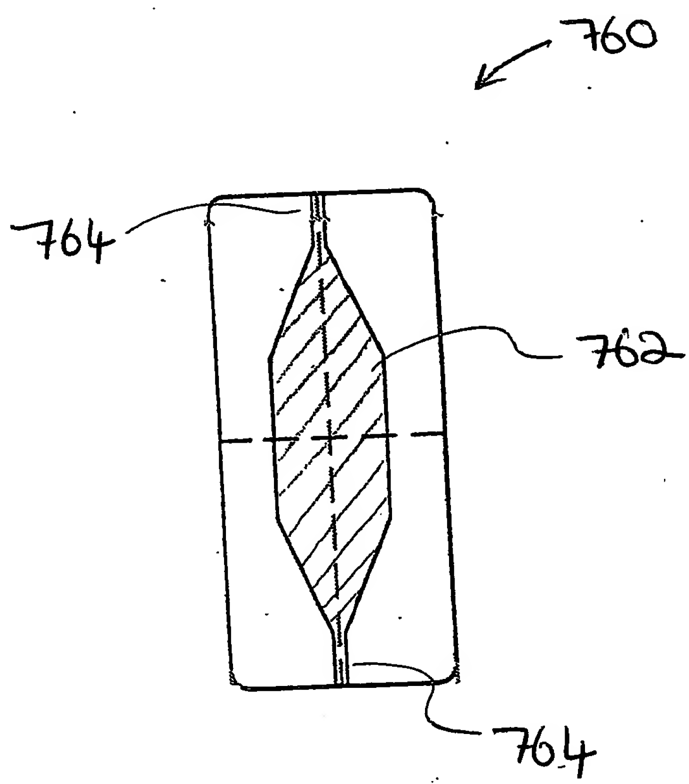


fig 7

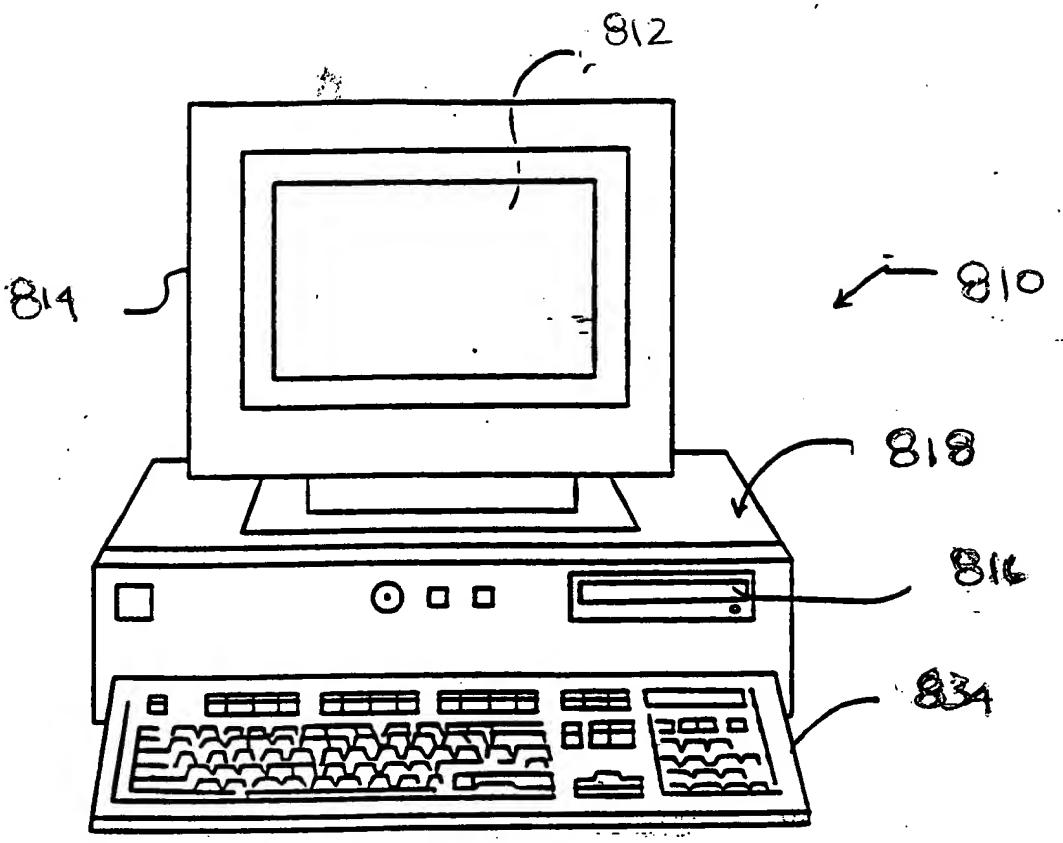


Fig 8A

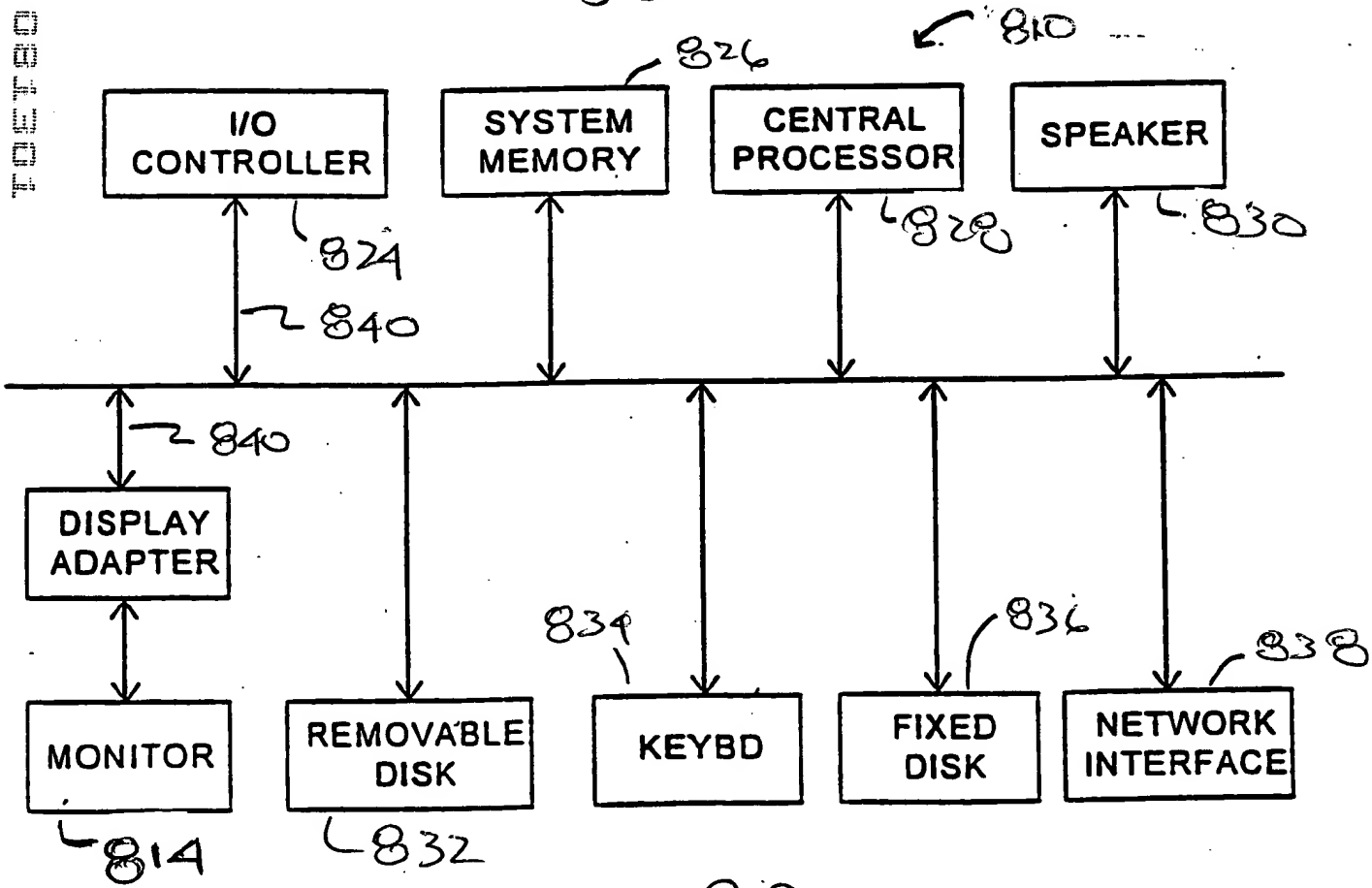
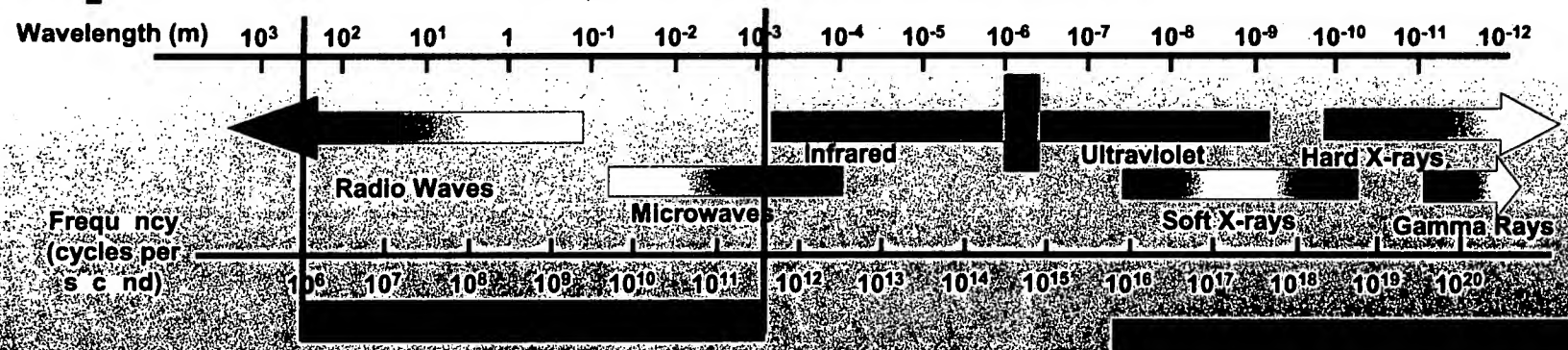


FIG. 8B

MCS: RF and Microwave



- **Detects protein "soft vibrations"**
  - *Protein Motions 10 psec – 100 nsec*
- **Complexation of Solvent**
  - Water, ions, cofactors, small molecules, other proteins

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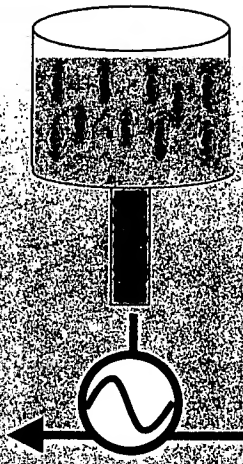
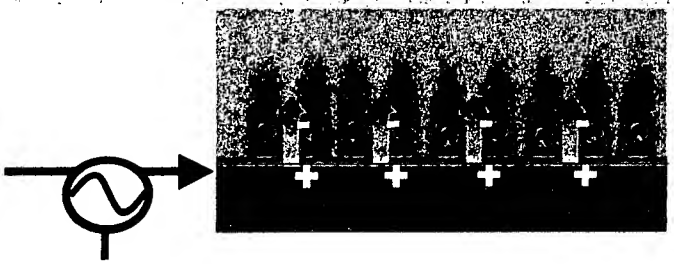
Integration of the Biology

- Biological systems as dielectric circuit element
- Integration into circuit configurations

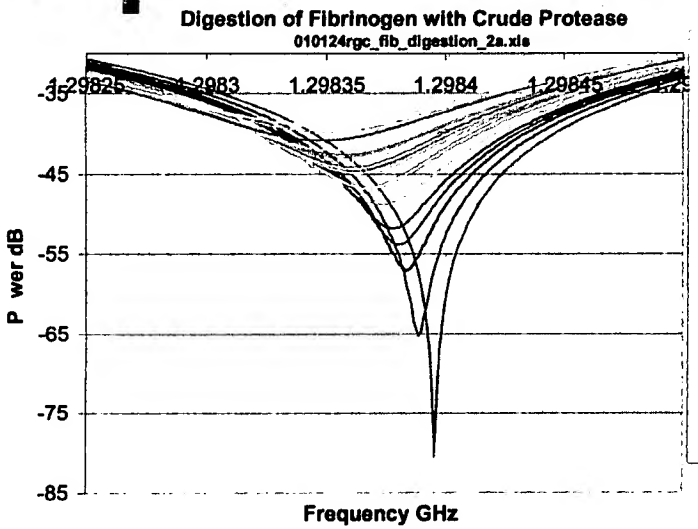


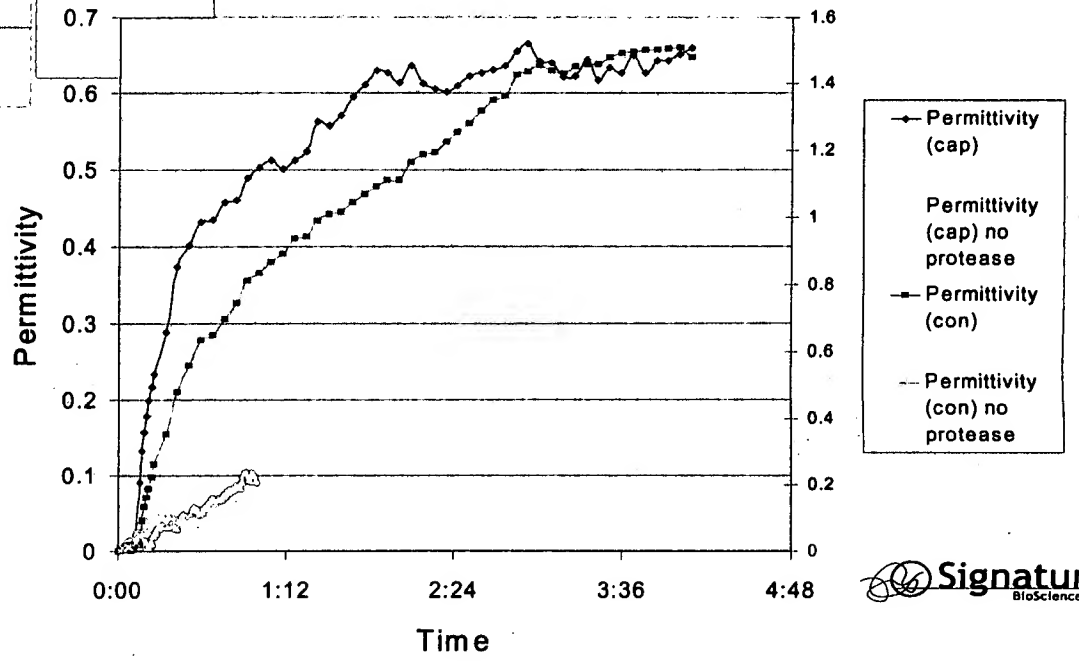
Solution-Phase



Solid-Phase

Permittivity vs. Structure:
Fibrinogen Digest





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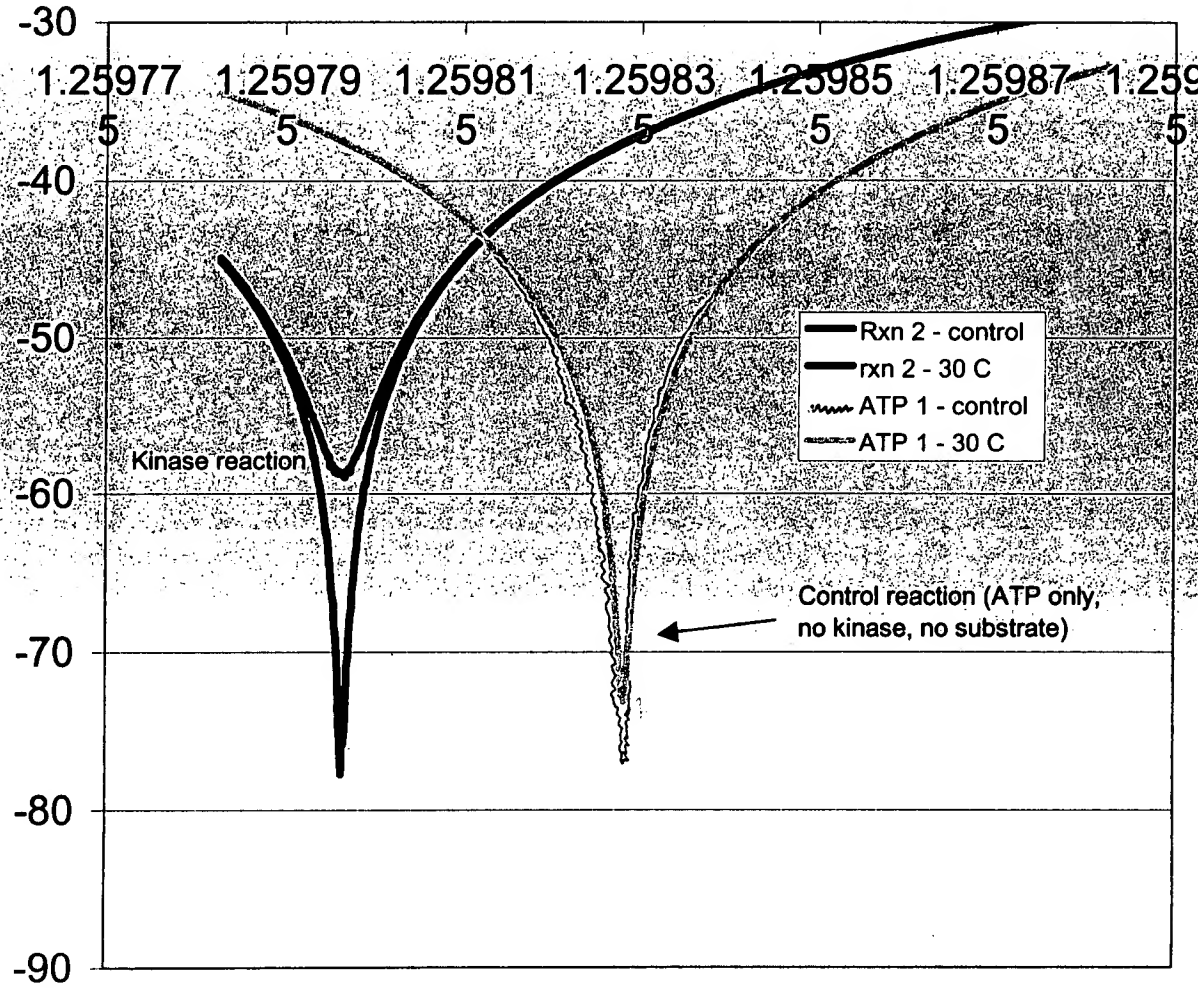
Tyrosine kinase assay

.132 units/ul c-src, 200 uM (.3 mg/ml) substrate (521) and 150 uM ATP



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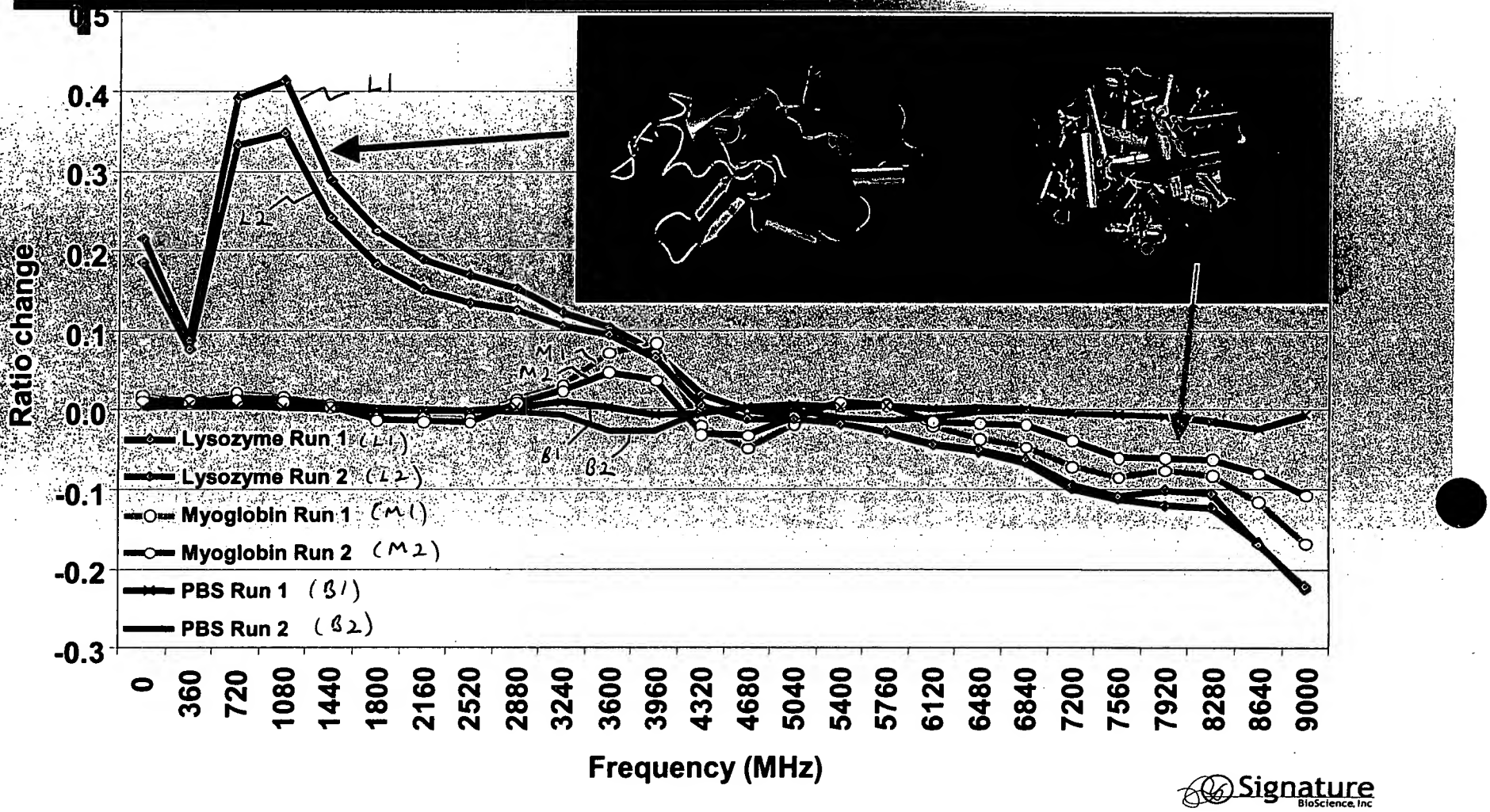
MCS broadband signatures

Differ between proteins



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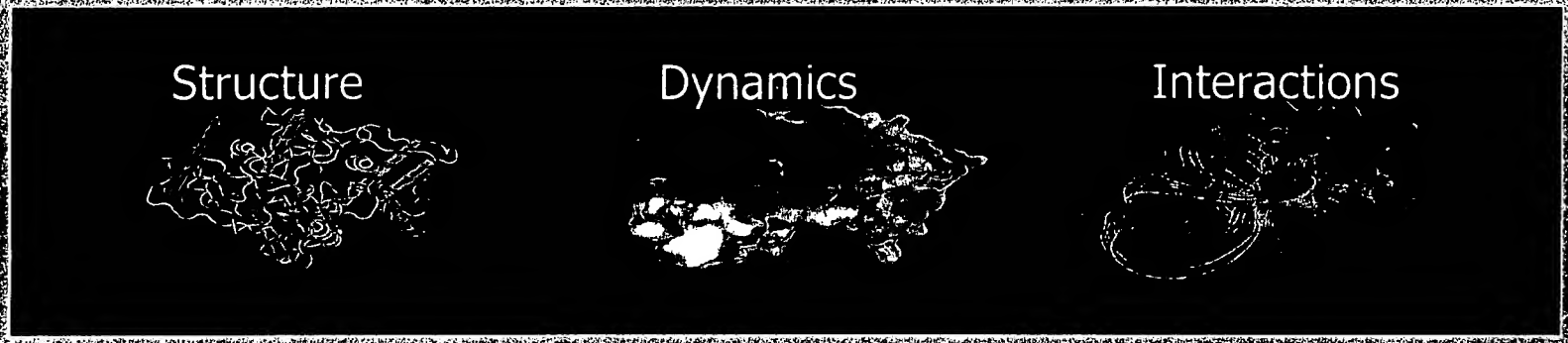
Value Proposition

- Permittivity→Function



- No Engineering→Direct and Rapid Access

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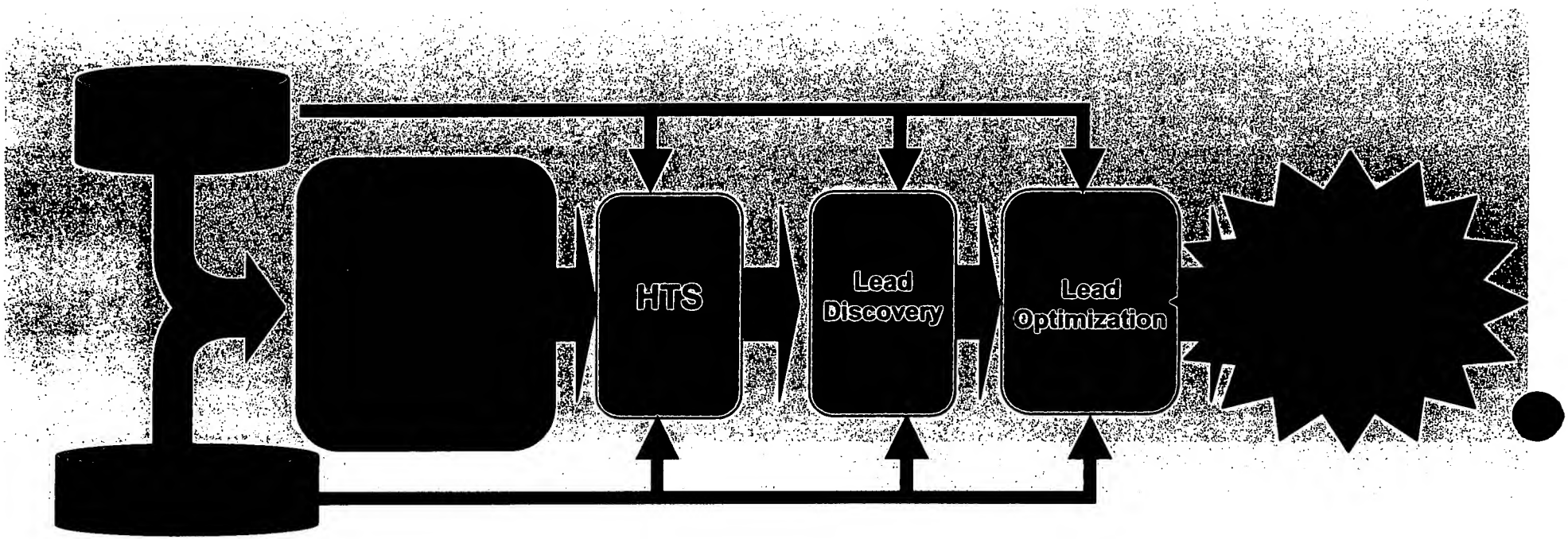
MCS in Drug Discovery:

A Parallel Approach



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MCS: solving discovery problems

- "Target-fishing"
  - we can detect proteins in solution
  - we can classify unknown protein targets
  - we can de-orphan unknown protein targets
- Quantifying binding
- Qualifying leads using protein/ligand classification with MCS
- SAR using MCS
- Cellular assays with MCS

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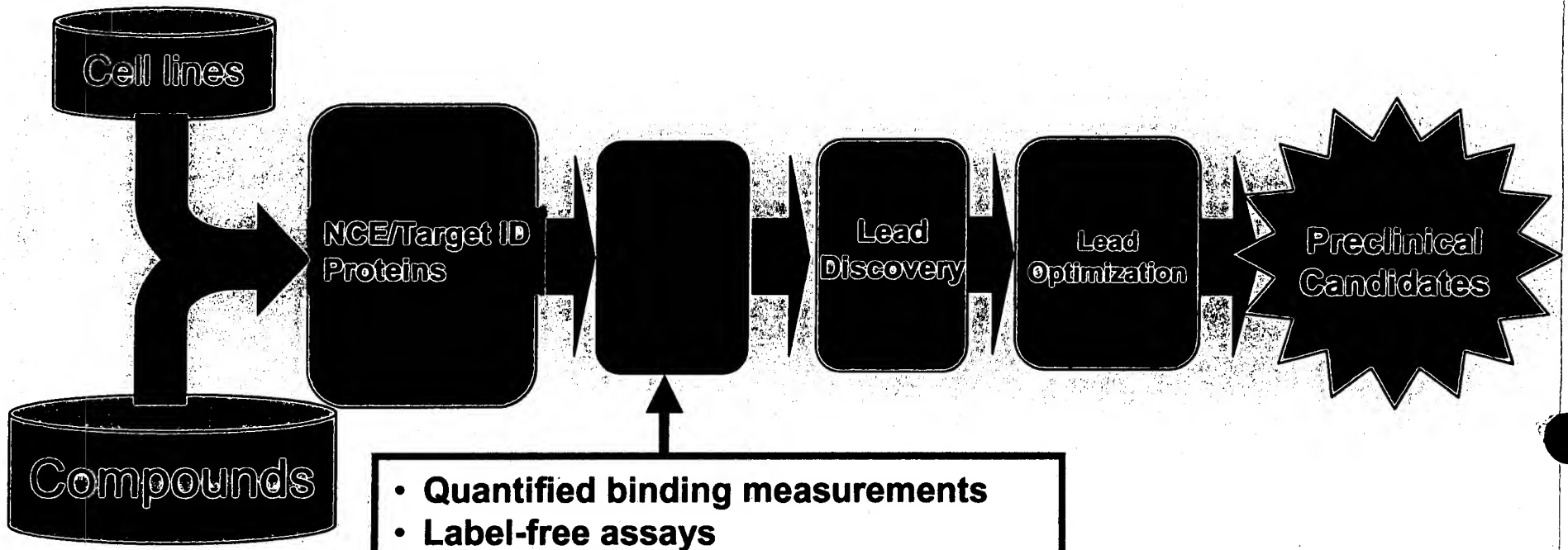
MCS in Drug Discovery



- **Quantified binding measurements**
- **Label-free assays**
- **Rapid assay prototyping and development**
- **Physiologically relevant conditions**
- **Medium throughput**
- **Molecular system**

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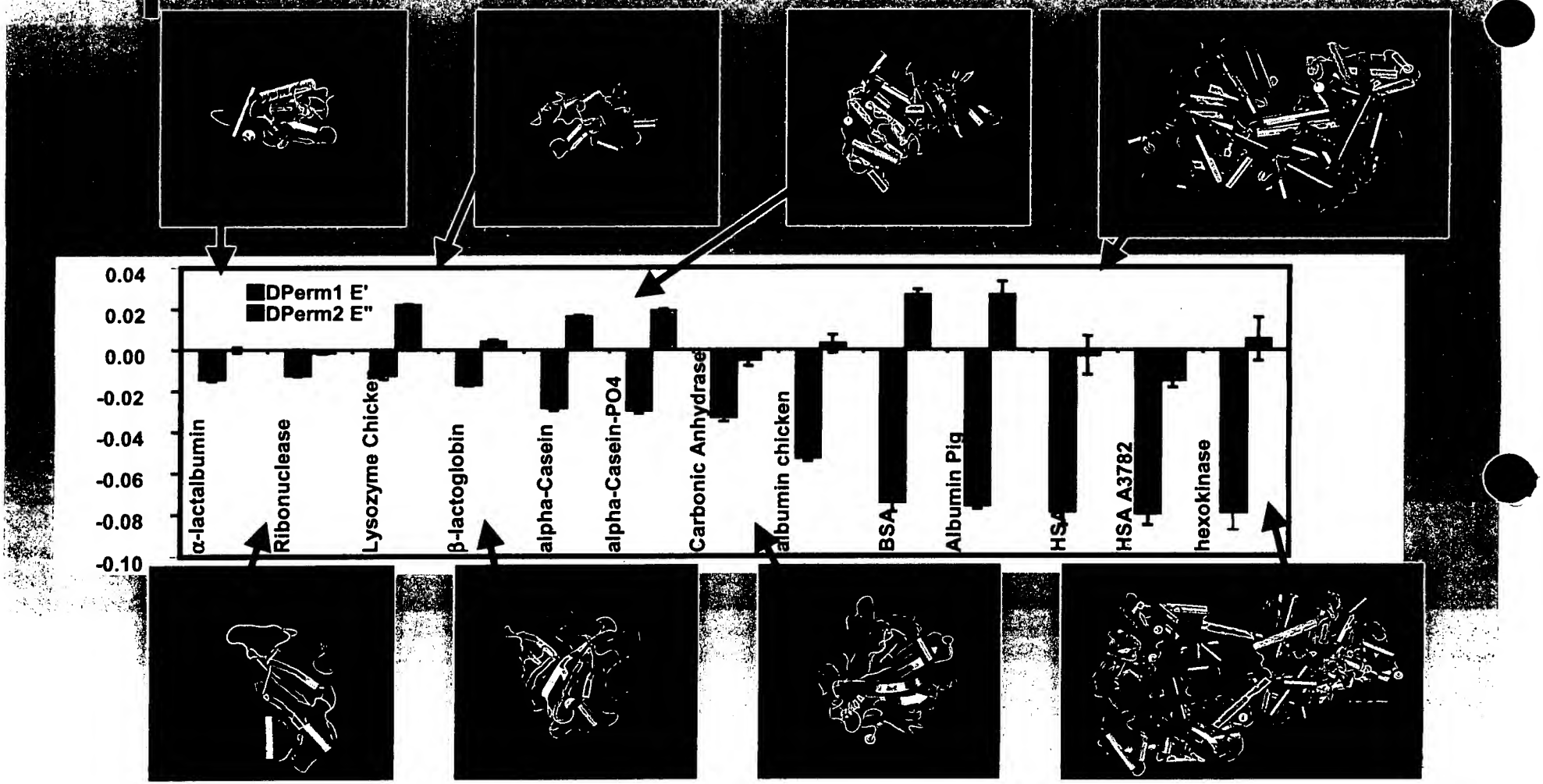
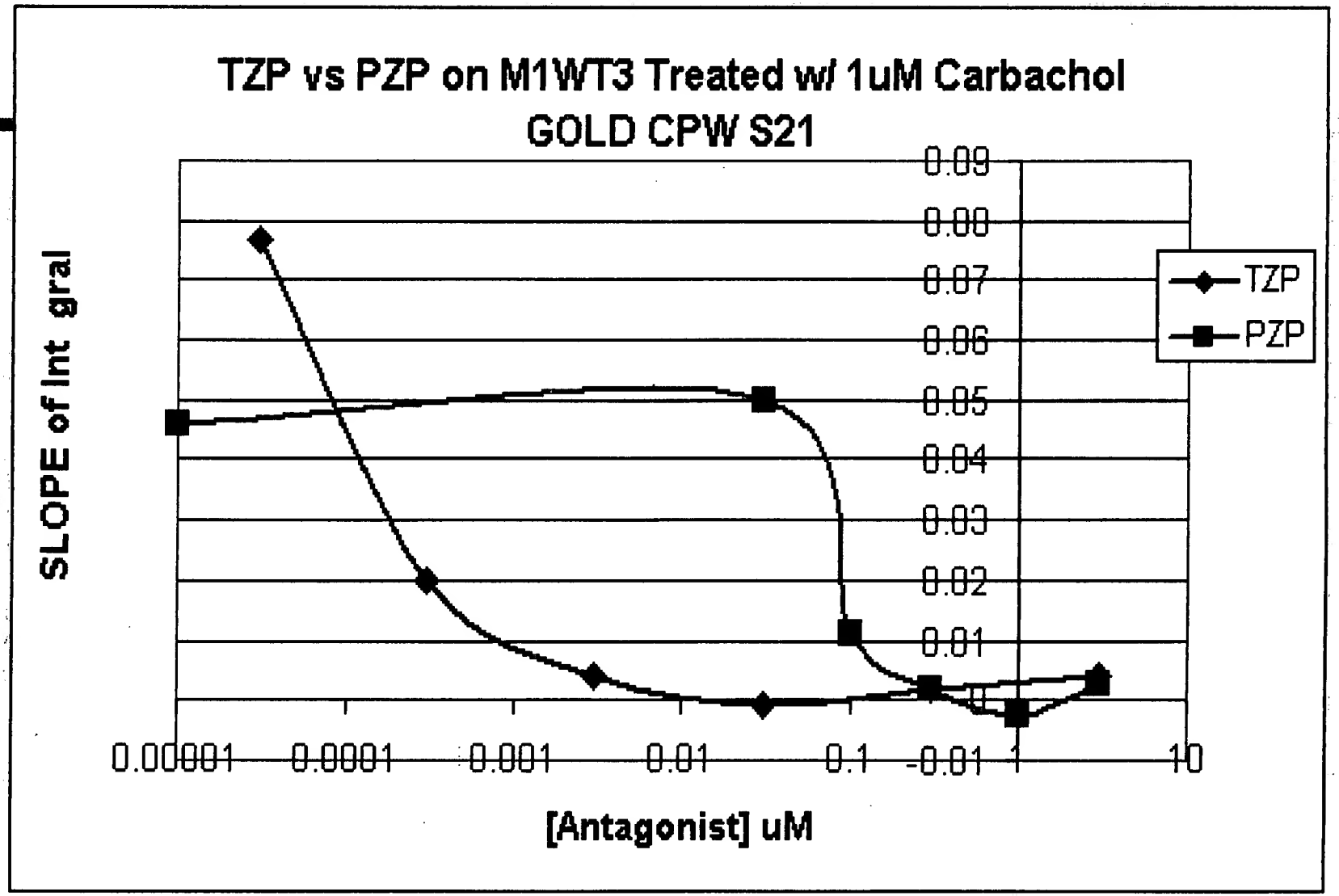
Similar proteins have similar signatures

Change in permittivity at 1.3 GHz





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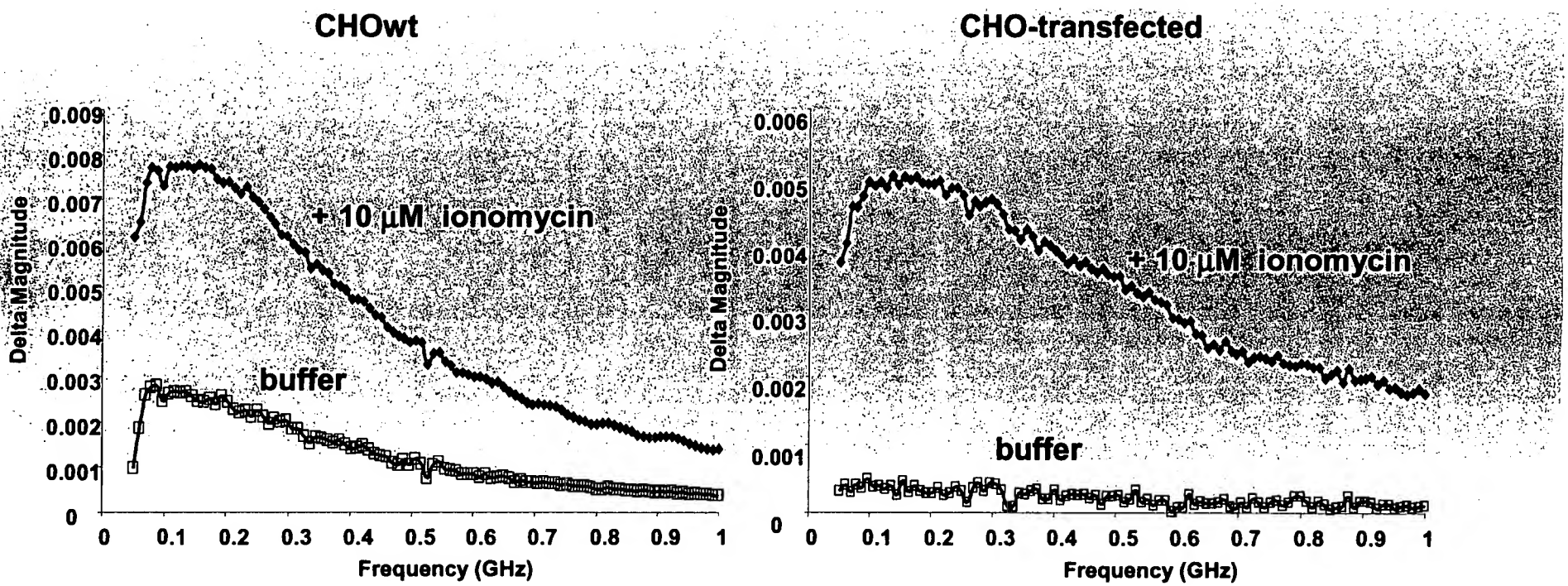
MCS cellular response to ionomycin



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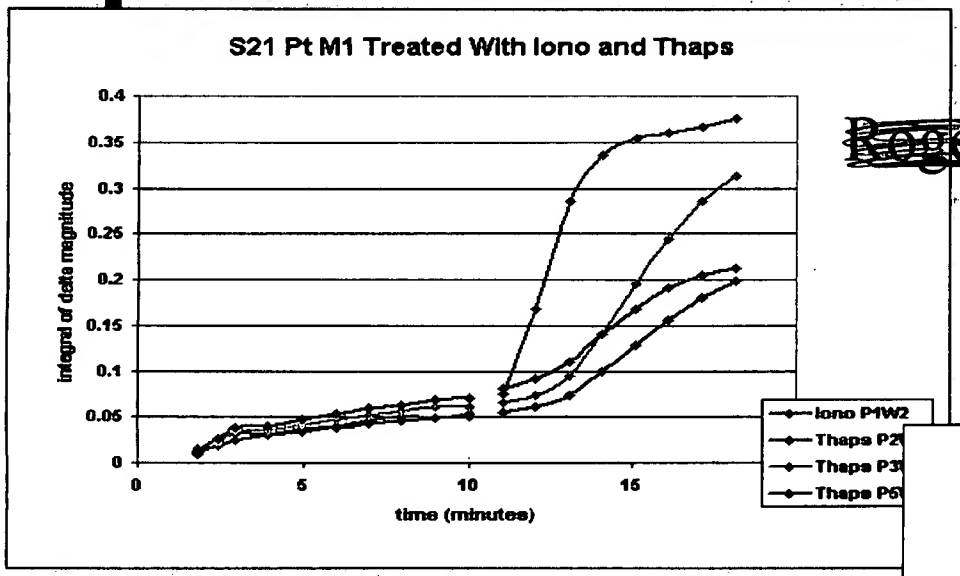
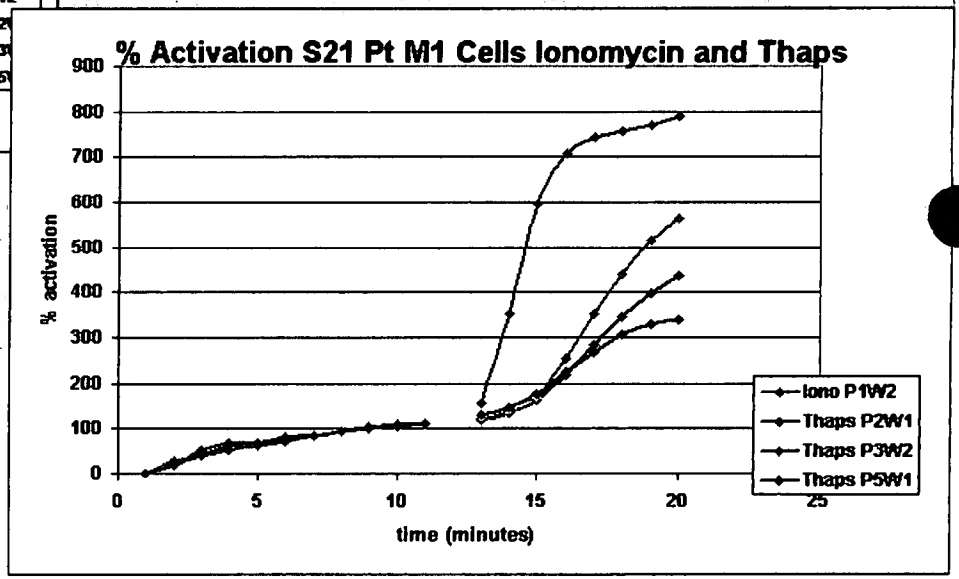
Thapsigargin



Roger Plot



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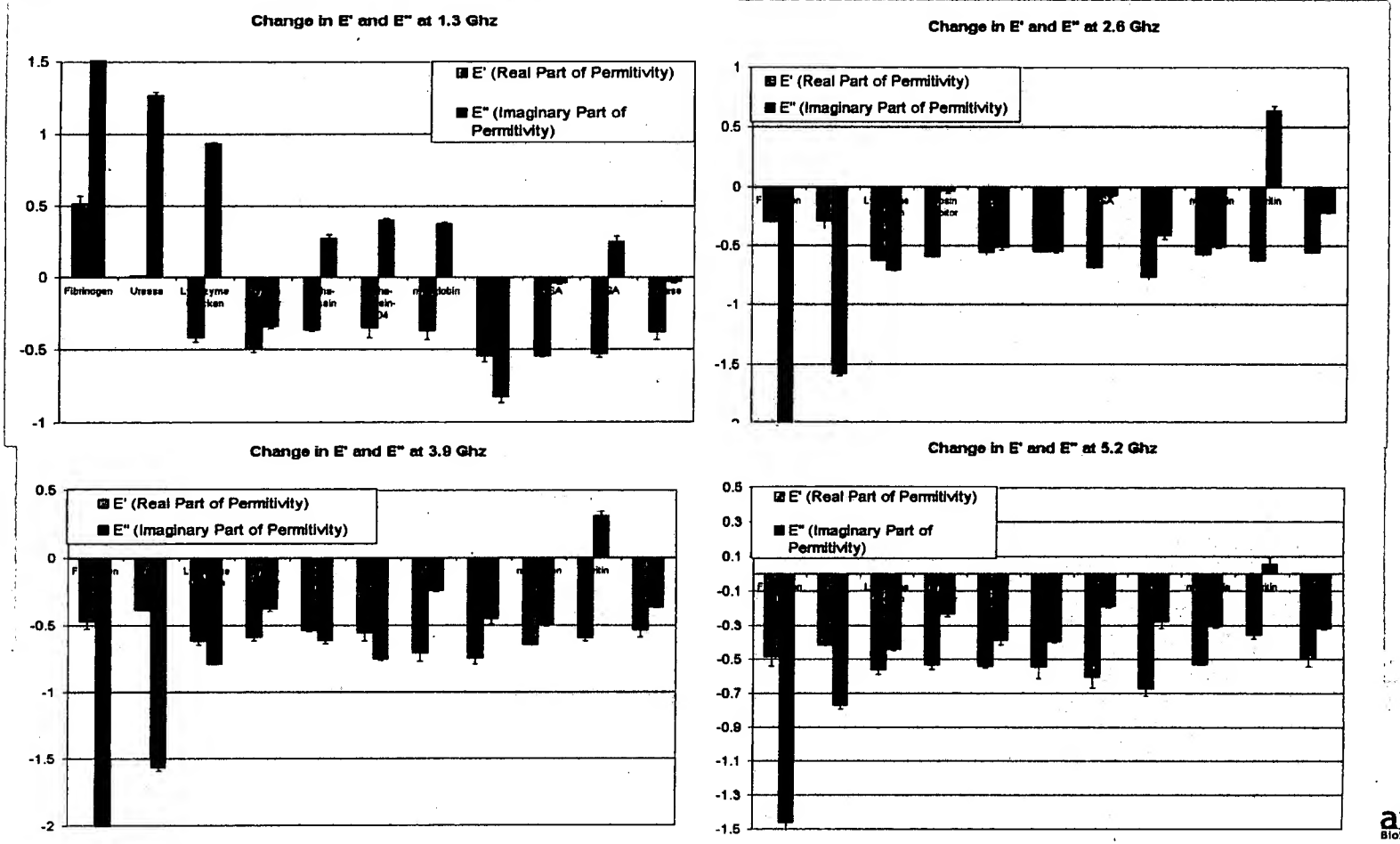
Multiple Discrete Frequency Analysis



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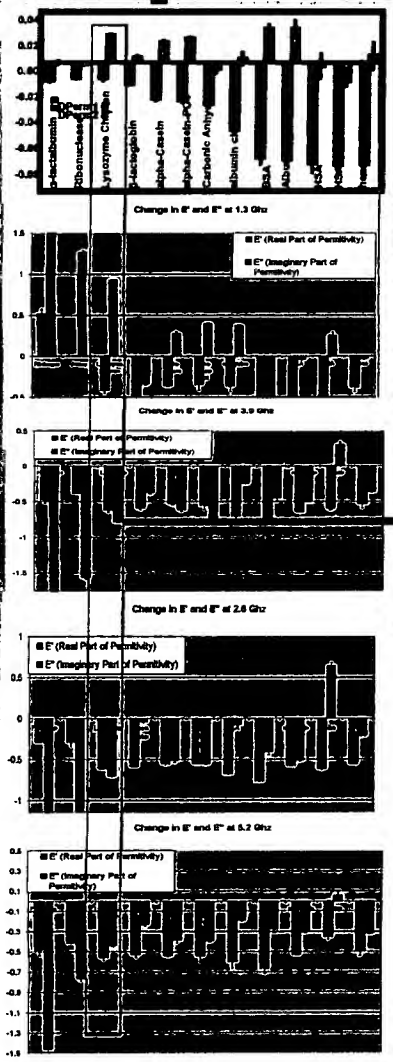
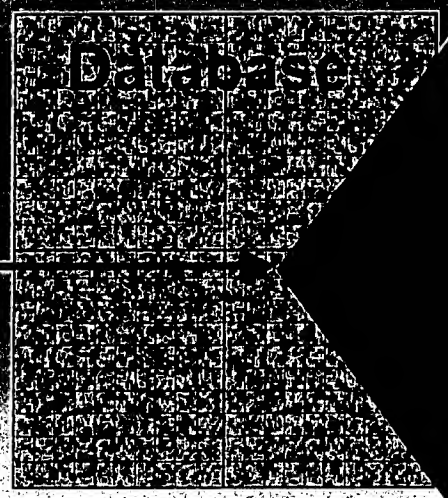
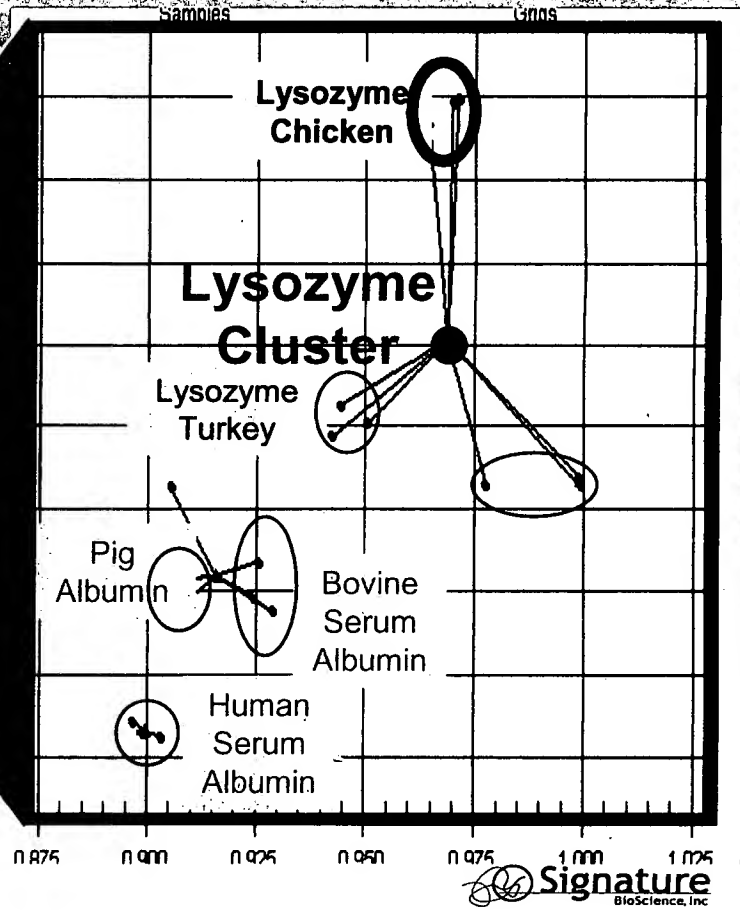
Tertiary structural homology prediction

(hypothetical)







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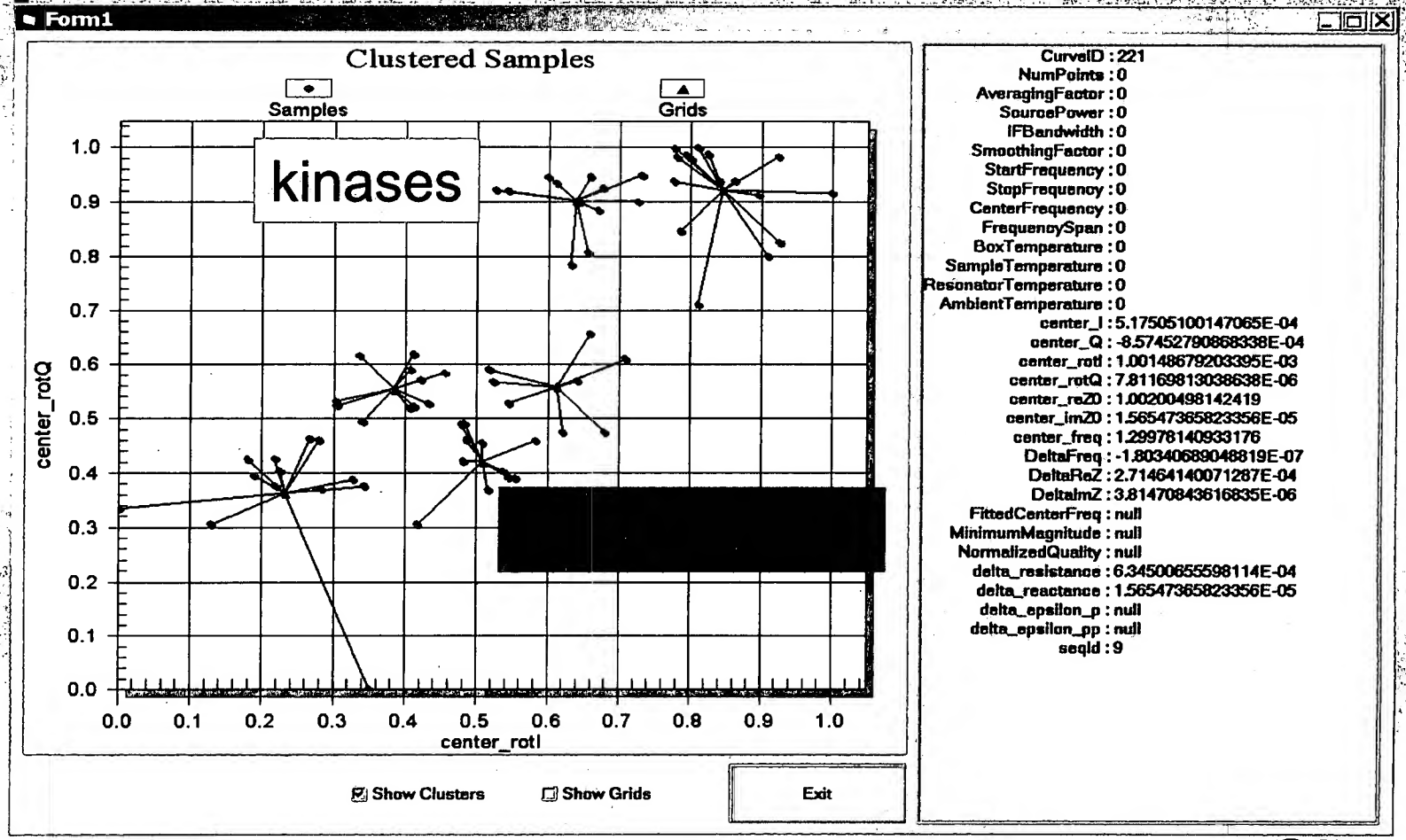
Clustering for protein function



(hypothetical)

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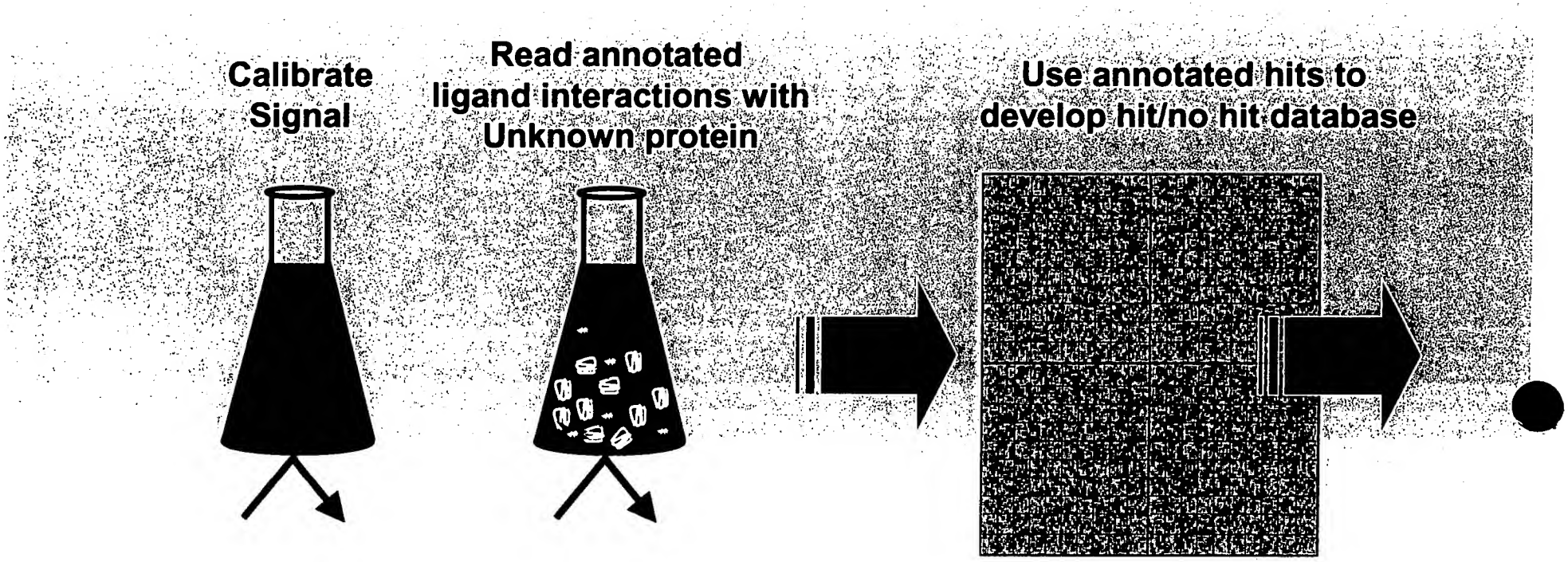
Or, de-orphaning using annotated compound libraries…



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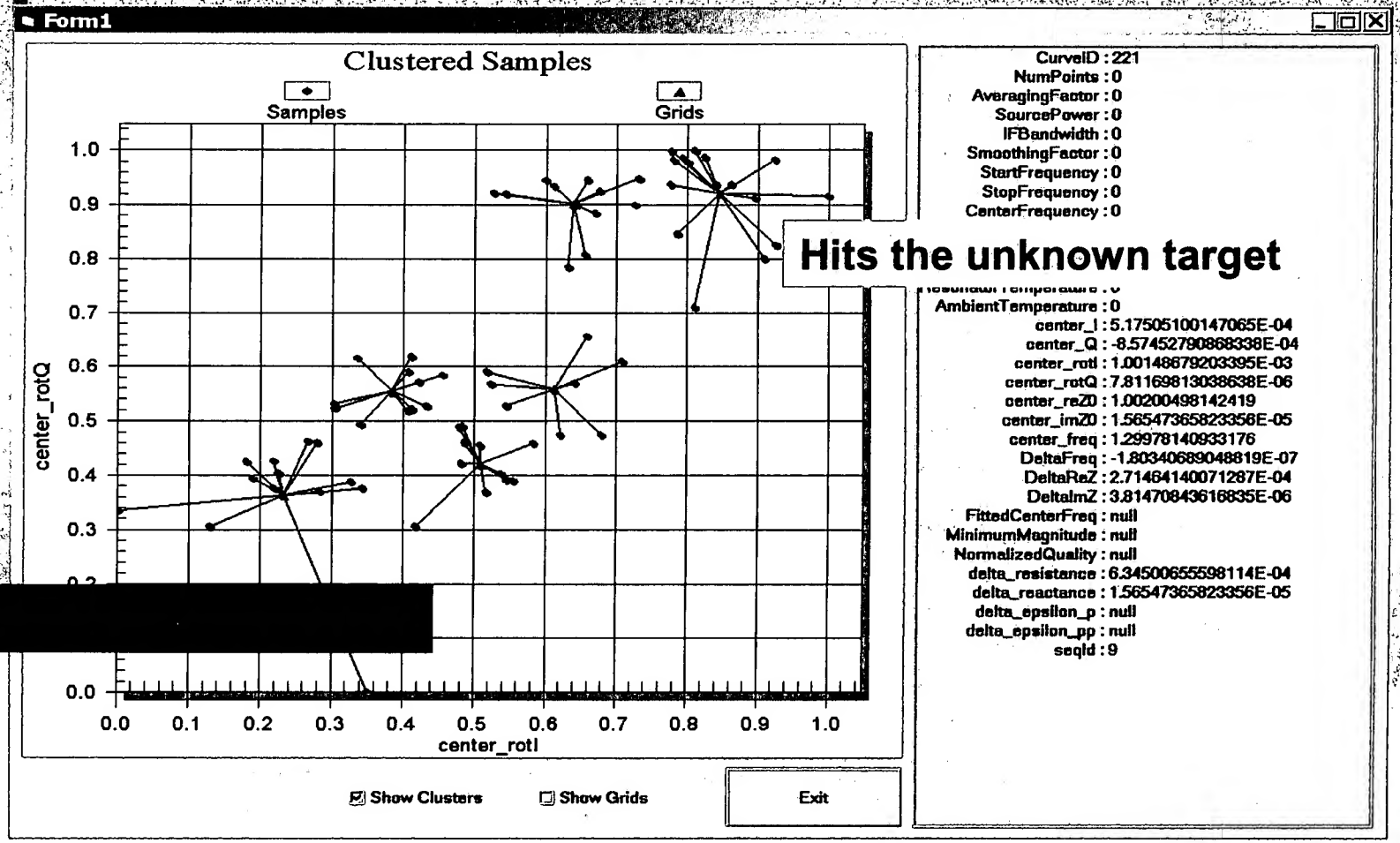
...Enabling clustering for compound effect

(hypothetical)



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Non-competitive binding assays

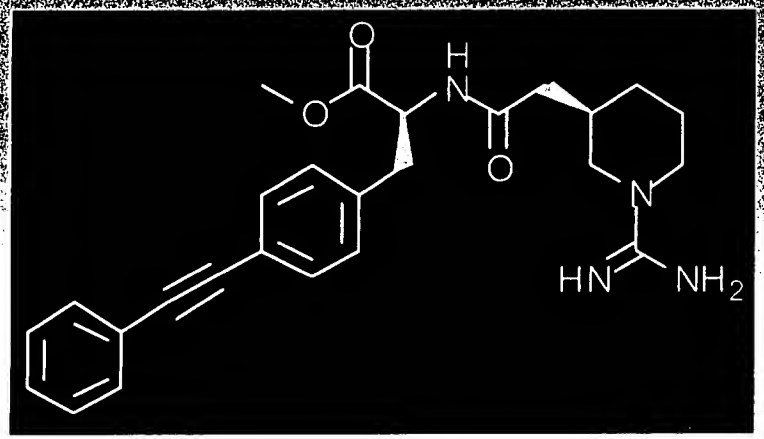
- Methods to detect weak binders are slow
  - Competitive assays usually won't work
- "Orphan-like" targets may have no affinity ligand
- Allosteric binders difficult to find
- Label artifacts
- Bioconjugation

IL-2/IL-2R Inhibitors

IL-2 is the principle cytokine involved in cell-mediated immunity.

Antibodies against IL-2Rα approved for graft rejection.

- Well-characterized small-molecule inhibitors of IL-2 have been discovered



IC_{50} = 3 μM

sunesis

Roche Research Center (Nutley)
J.W. Tilley, et al. JACS (1997) 119, 7589-7590.

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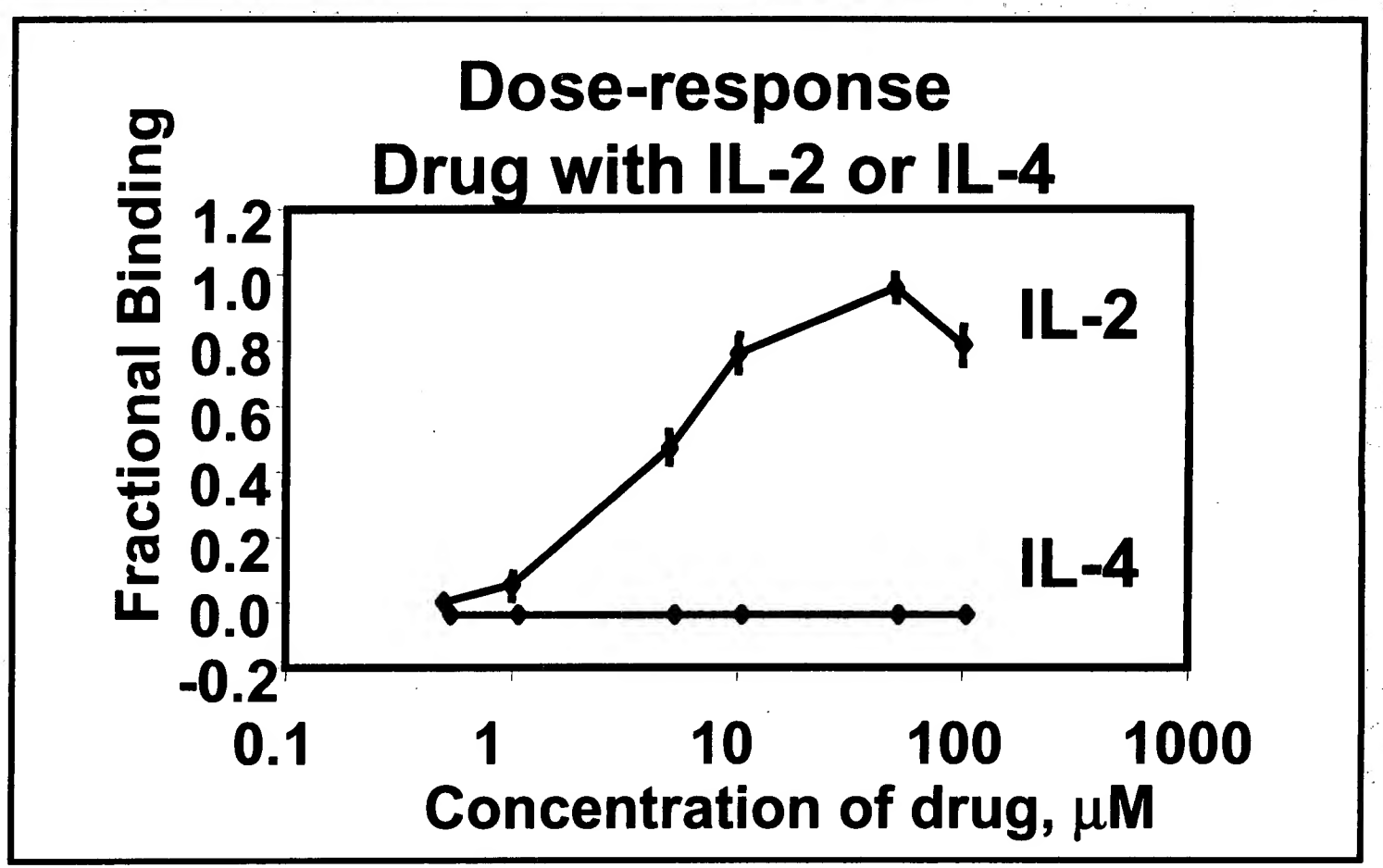
MCS analysis of binding to IL-2, IL-4



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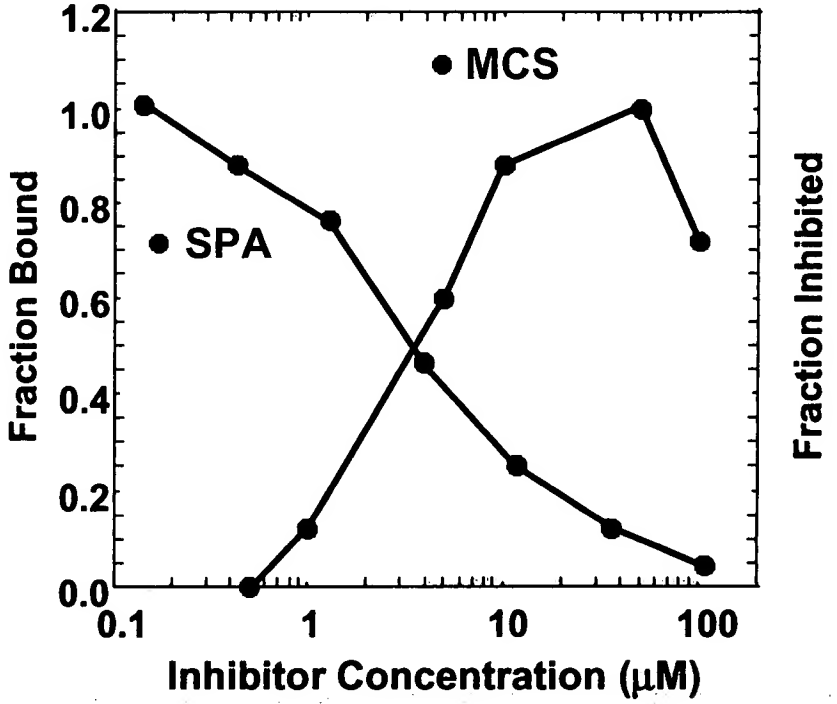
MCS binding results same as others

Method	IC_{50}/K_d
SPA	3 μM
MCS	***4 μM***
AUC	5 μM
SPR	20 μM
ITC	4 μM



SPA – scintillation proximity assay
MCS – multipole coupling spectroscopy
AUC – analytical ultracentrifugation
SPR – surface plasmon resonance
ITC – isothermal calorimetry

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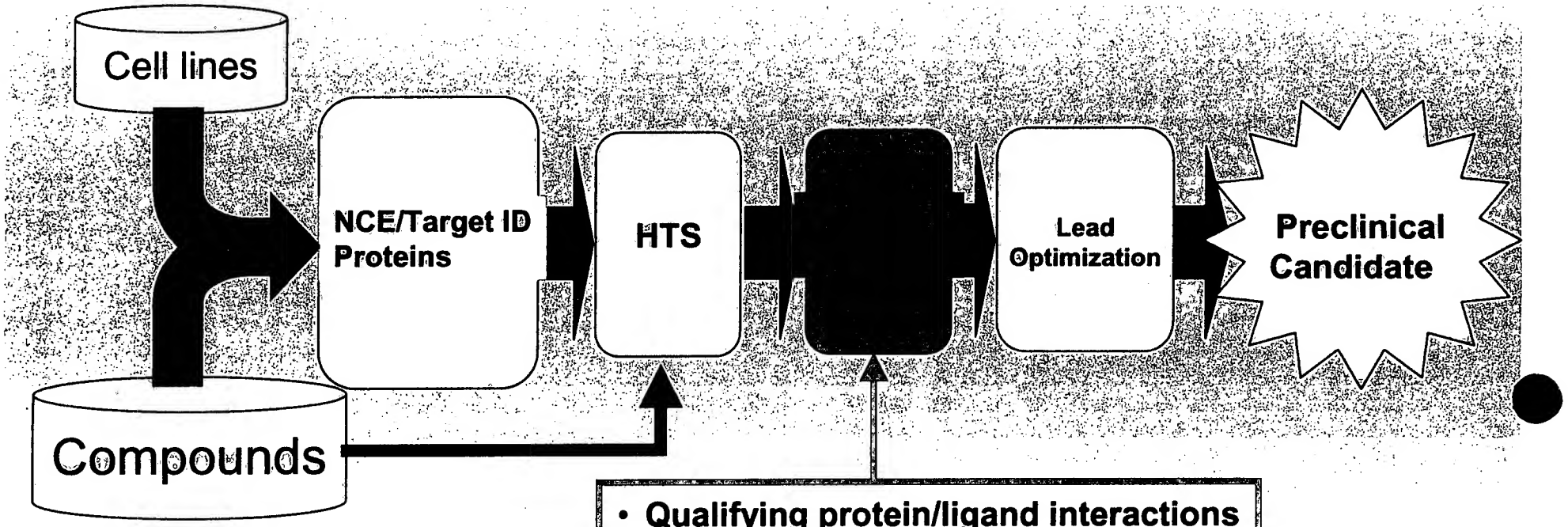
MCS in Drug Discovery

Drug Discovery Process



- Qualifying protein/ligand interactions
- Determine binding IC_{50}s
- Selectivity against relevant targets
- Eliminate non-drug like compounds
- Molecular system, some cellular

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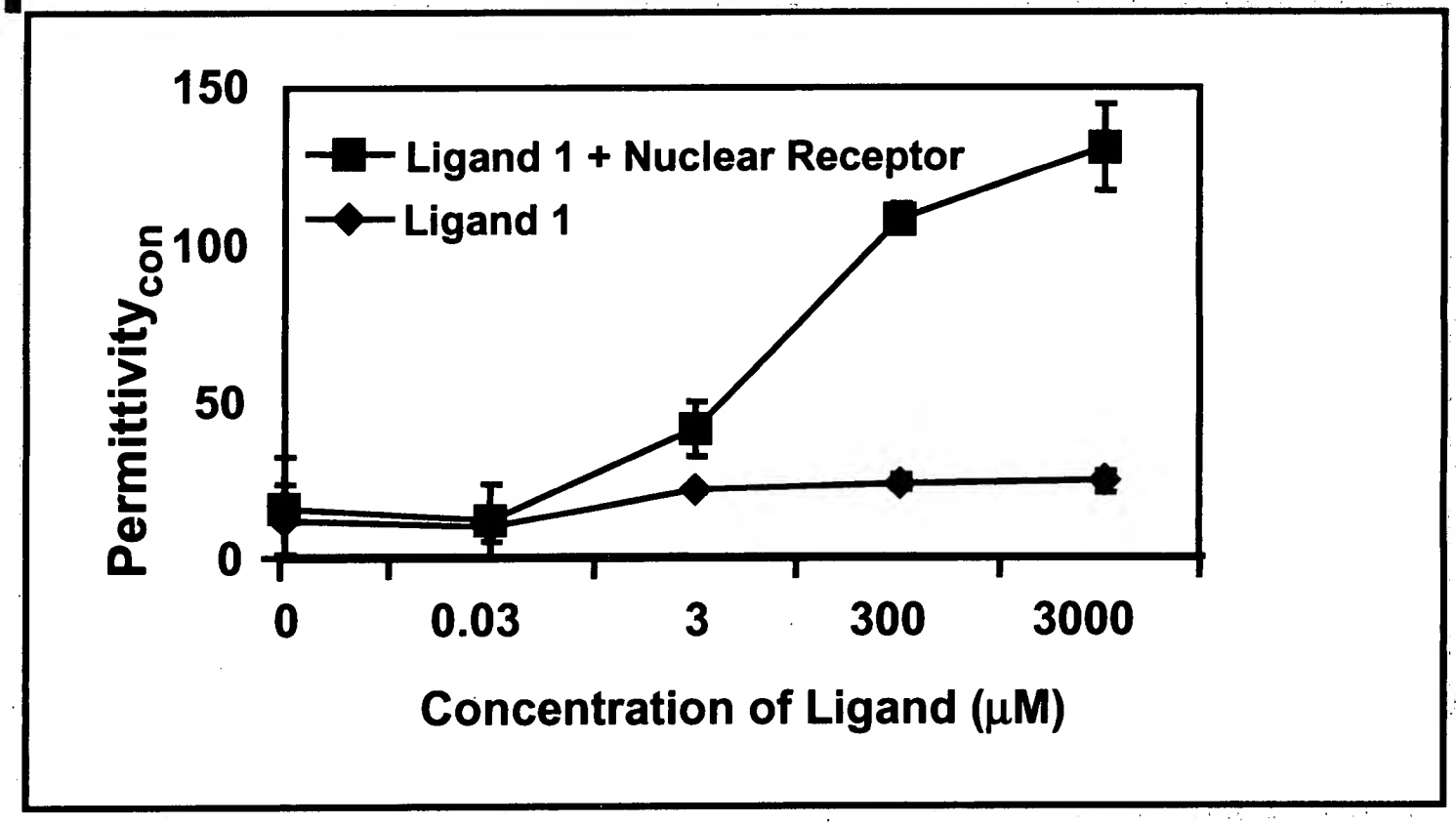
Ligand function classification

- "Bin" hits
  - agonists would cause similar responses to each other
    - distinct responses from antagonists
- Nuclear Receptor-based
  - "binning" of hits
  - quantify relationships to known compounds
    - e.g. Ligand-1 like or Ligand-2 like

Lack of a functional readout is a problem

- No ready, quick method for categorizing the effect a "hit" chemical has on a given target, when certain profiles are desired (ie, a functional, but not chemical, copy)
- Clear desire for a fast means of "target-fishing" using annotated compound libraries and other techniques

MCS of NR – L1 interaction at 1.3 GHz



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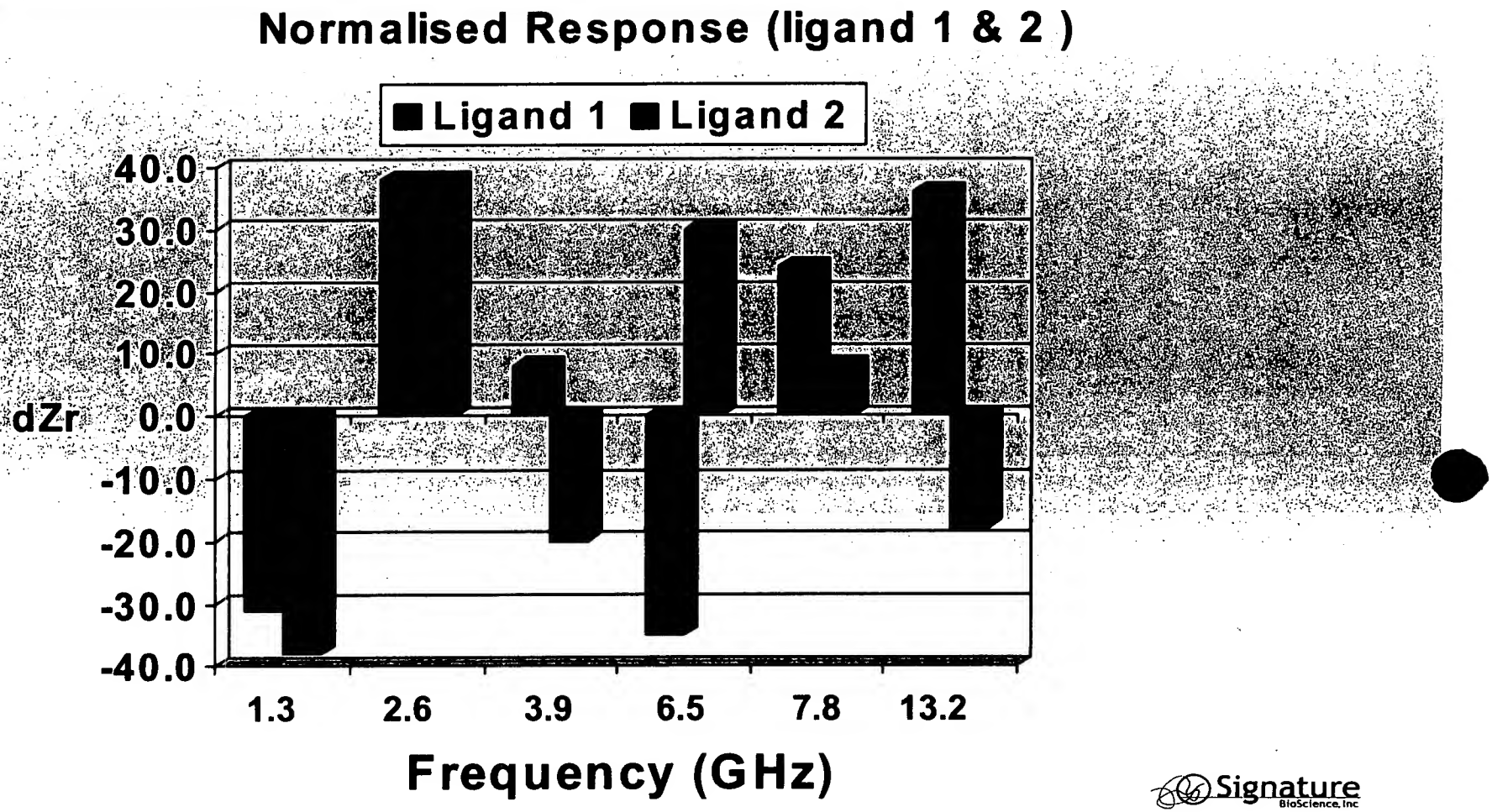
NR/ligand interaction comparison



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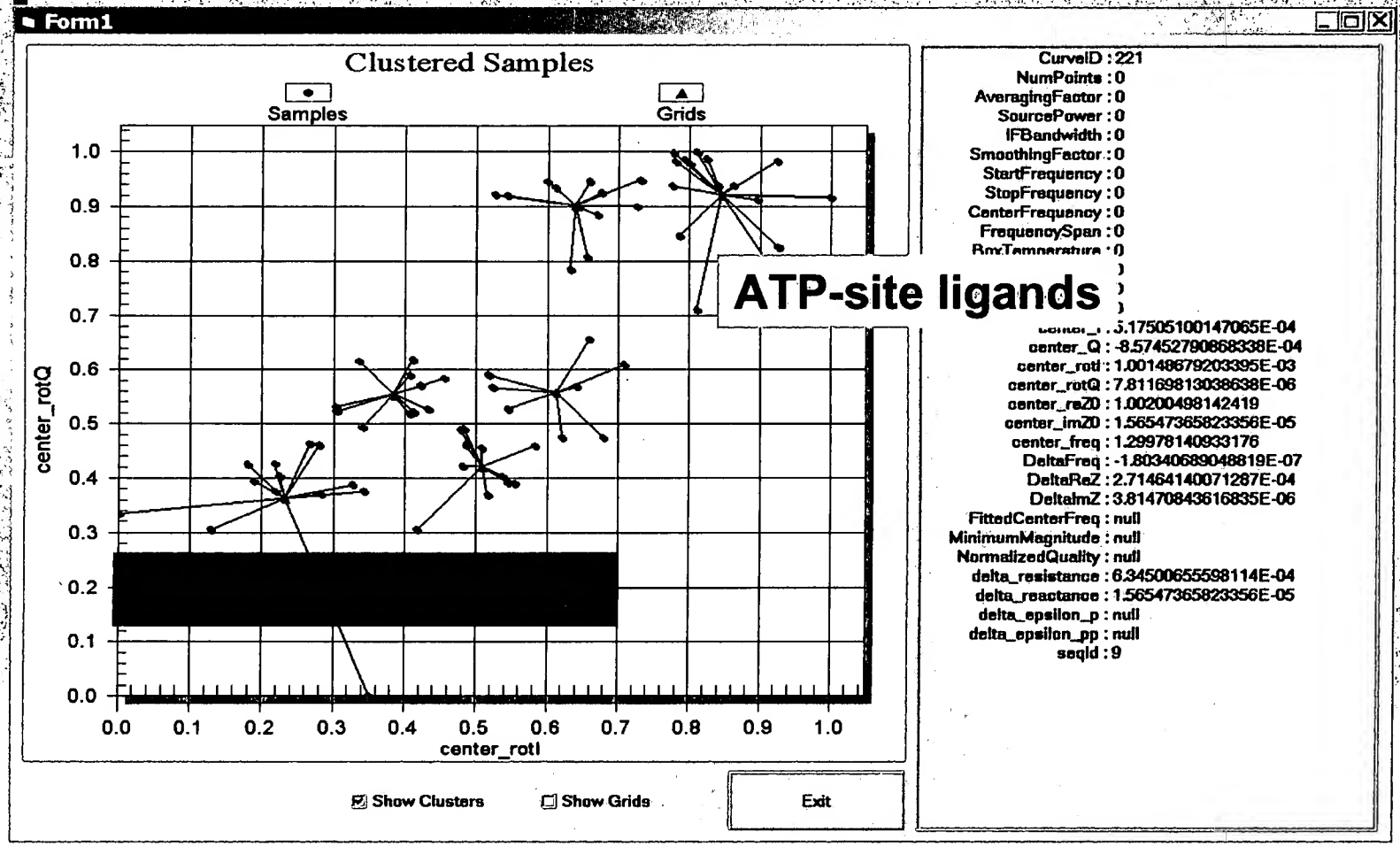
...Enabling clustering for ligand function (hypothetical)



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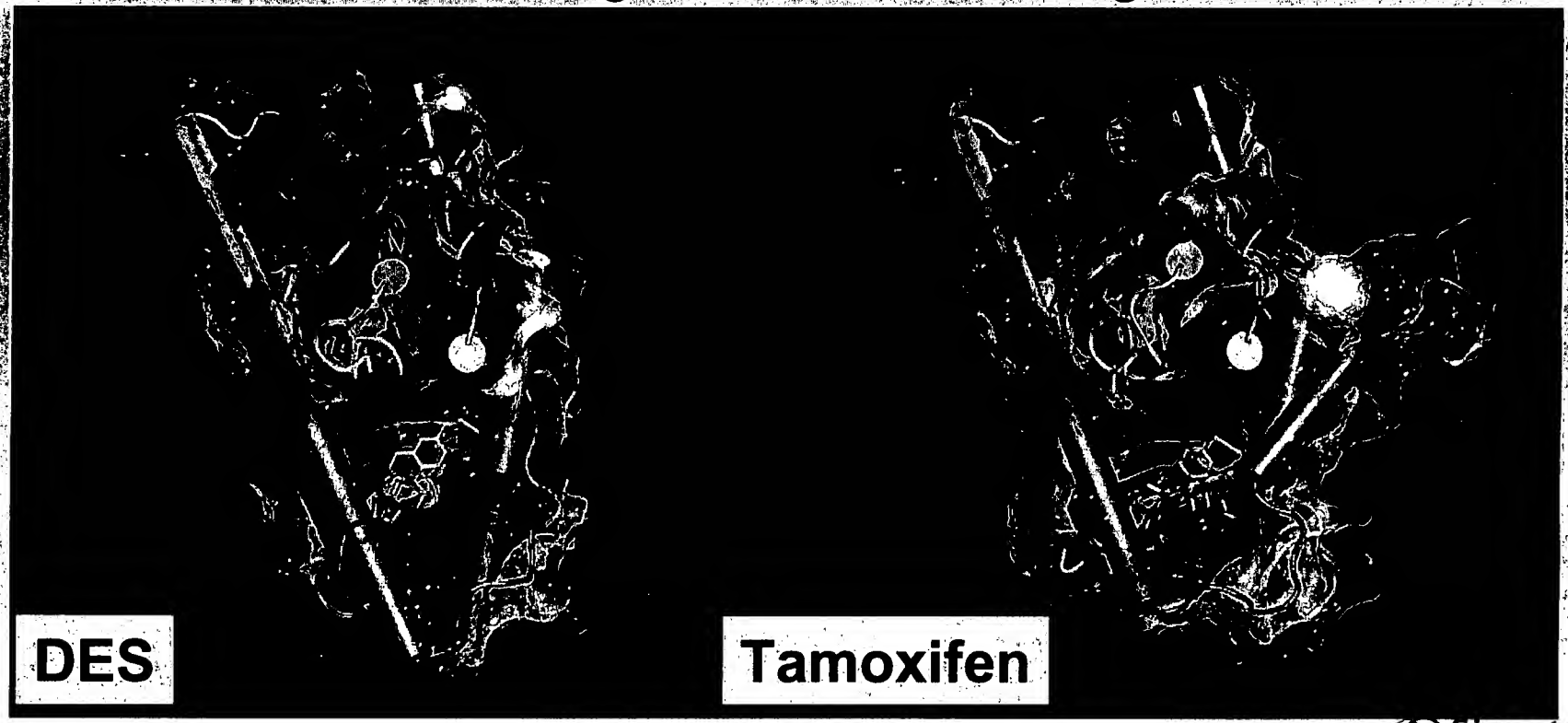
Structure/activity using MCS ?

- The opportunity:
- Perform X-ray crystallography or NMR routinely
- Earlier in the discovery process
- The problem:
- Cost, reagents required, technology repertoire limitations, and time-consuming nature of the processes involved, are prohibitive

Protein Function: Estrogen receptor-ligand interaction

- X-ray analysis has shown that DES (agonist) and Tamoxifen (antagonist) cause subtly different conformation changes to ER on binding interaction



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MCS signatures correlate interaction data

SAR Data from ER Model System

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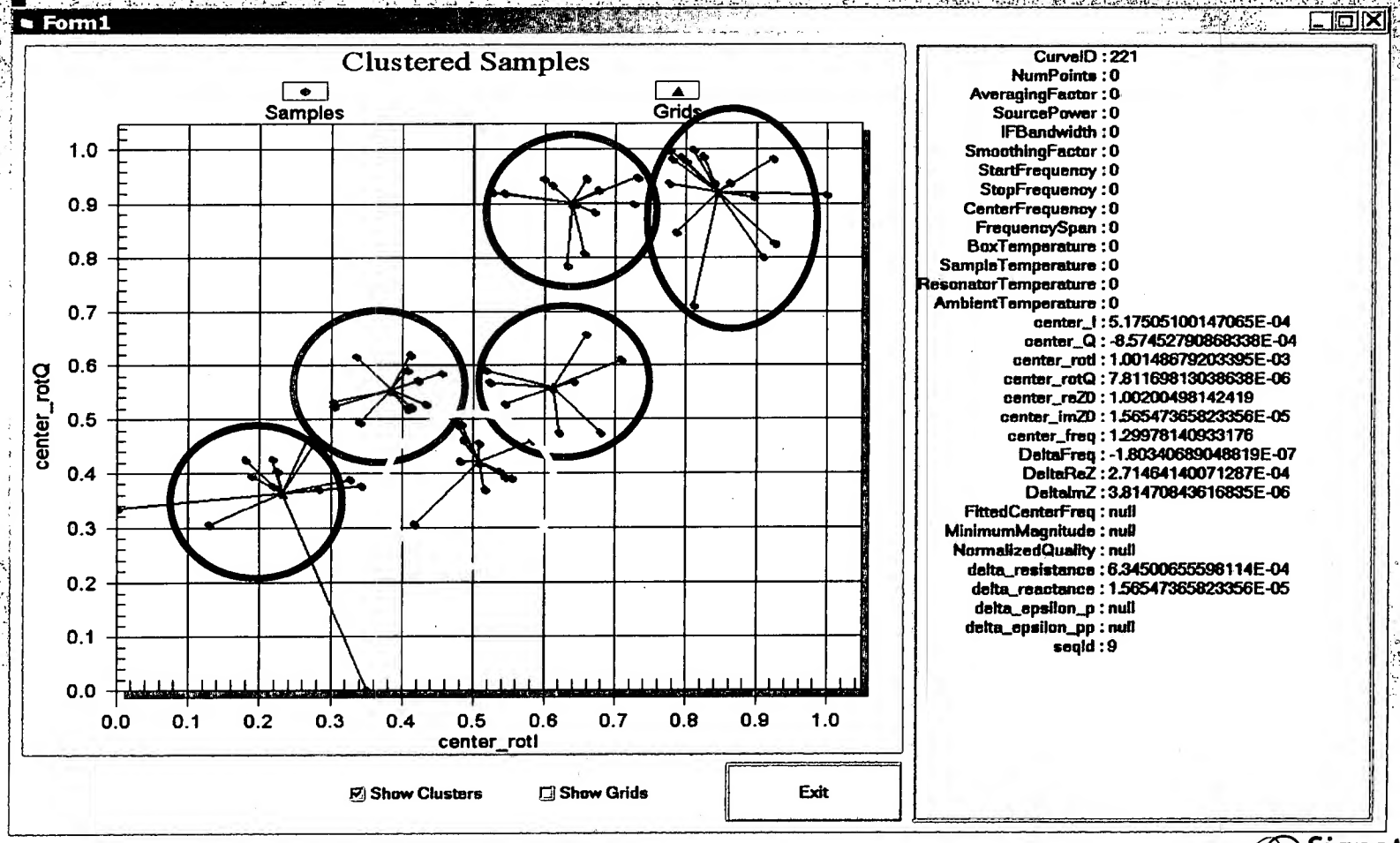
SAR with MCS – x-ray in advance

- Obtaining predicted structural readouts, enabled by "wet-lab" MCS data, and augmented by unique software…
  - Jump starts SAR, typically undertaken later

...Enabling clustering for ligand function (hypothetical)



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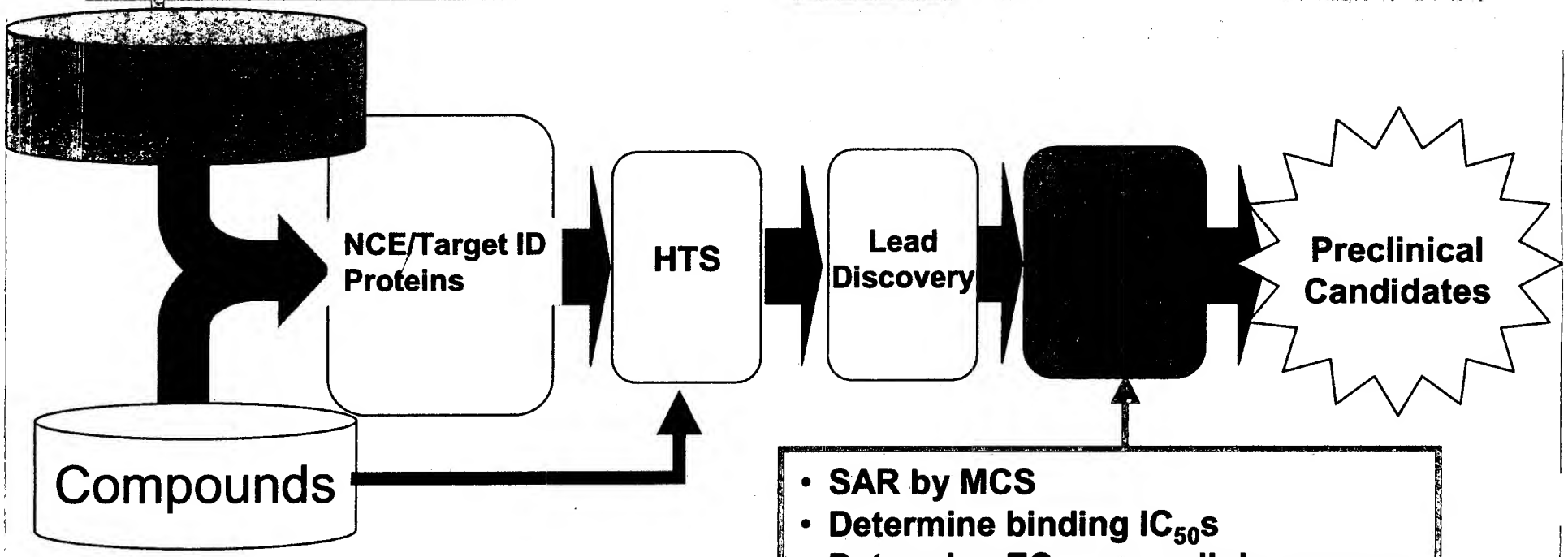
MCS in Drug Discovery



- SAR by MCS
- Determine binding IC_{50}s
- Determine EC_{50}s on cellular assays
- Eliminate compounds for ADMET problems
- Determine specificity
- Cellular systems, some molecular

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MCS: solving discovery problems

- "Target-fishing"
  - we can detect proteins in solution
  - we can classify unknown protein targets
  - we can de-orphan unknown protein targets
- Quantifying binding
- Qualifying leads using protein/ligand classification with MCS
- SAR using MCS
- Cellular assays with MCS

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Cellular MCS: Overview

- Protein structure→cell organization
- Many physiologic processes can be measured
  - GPCR-mediated pathway induction
  - Ion channel modulation
  - Morphologic changes
  - Apoptotic events

Cellular MCS

- Protein Structure→Cellular Organisation
- MCS Measures Physiologic Changes in Cells
  - Ion Flux
  - Cytosolic cAMP/Ca2+
  - Morphologic Changes
  - Membrane changes

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Specificity in MCS Cellular Analyses

- Spectral Response
- Kinetics
- "Orthogonal" properties
  - Protein expression levels
  - Focused libraries
  - Diverse cell populations

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MCS hits major screening bottlenecks…

- Target ID, validation, *access* ✓
- Rapid Assay Development ✓
- Secondary Screening and Lead Optimization ✓
- Data Management and Analysis ✓

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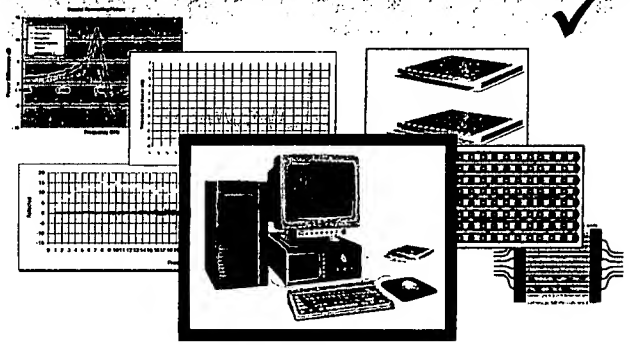
...and MCS meets defined "drivers" for new detection technologies

- Simple one step homogeneous assay ✓
- Avoid radioactivity, safety, disposal costs ✓
- Sensitivity to replace radioactivity ✓
- Reagent, target and compound sparing ✓
- Speed / throughput ✓
- Higher quality information ✓



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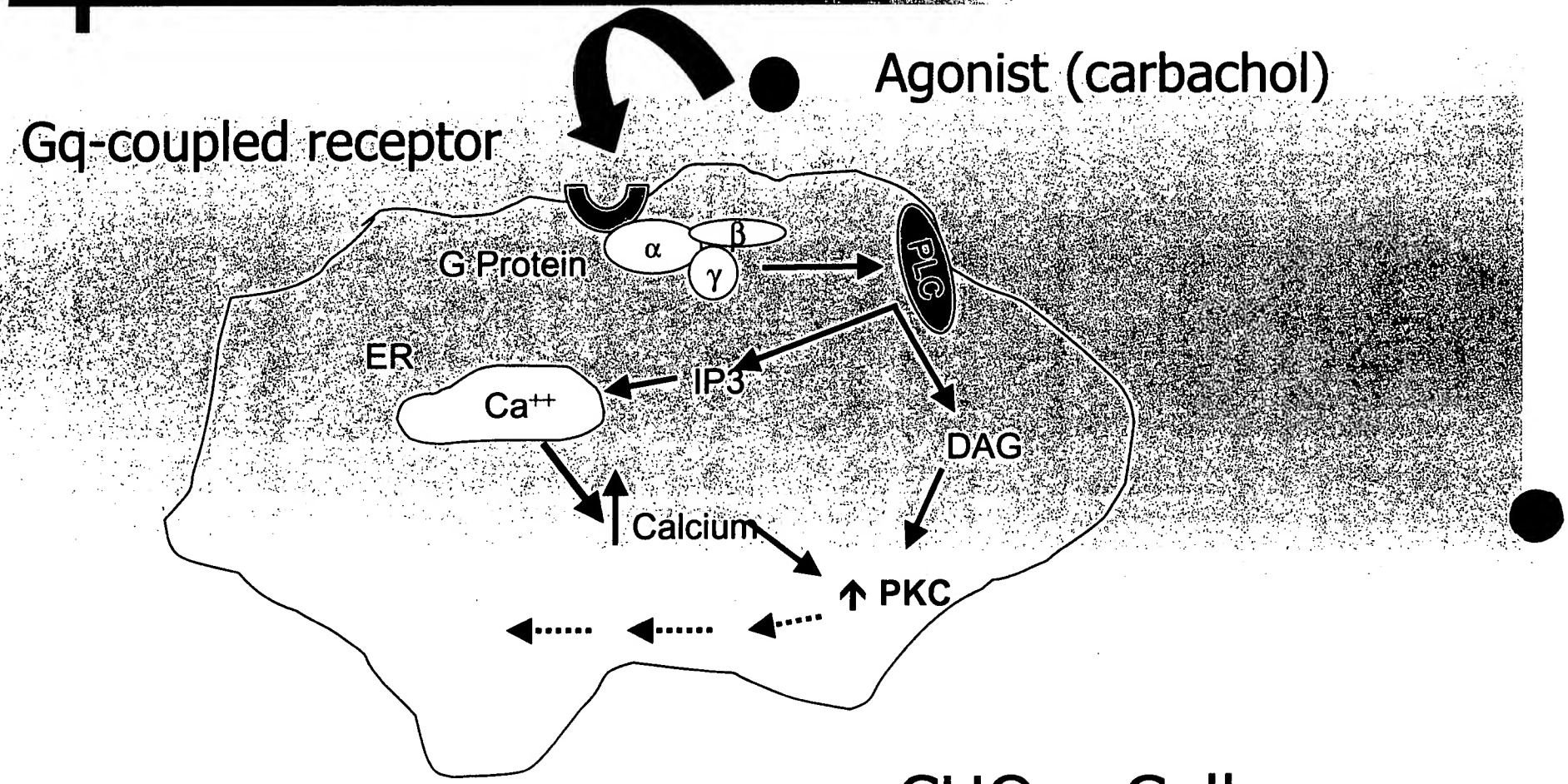
A GPCR-mediated pathway:

Activation of muscarinic m_1 receptor



CHO_{m1} Cell

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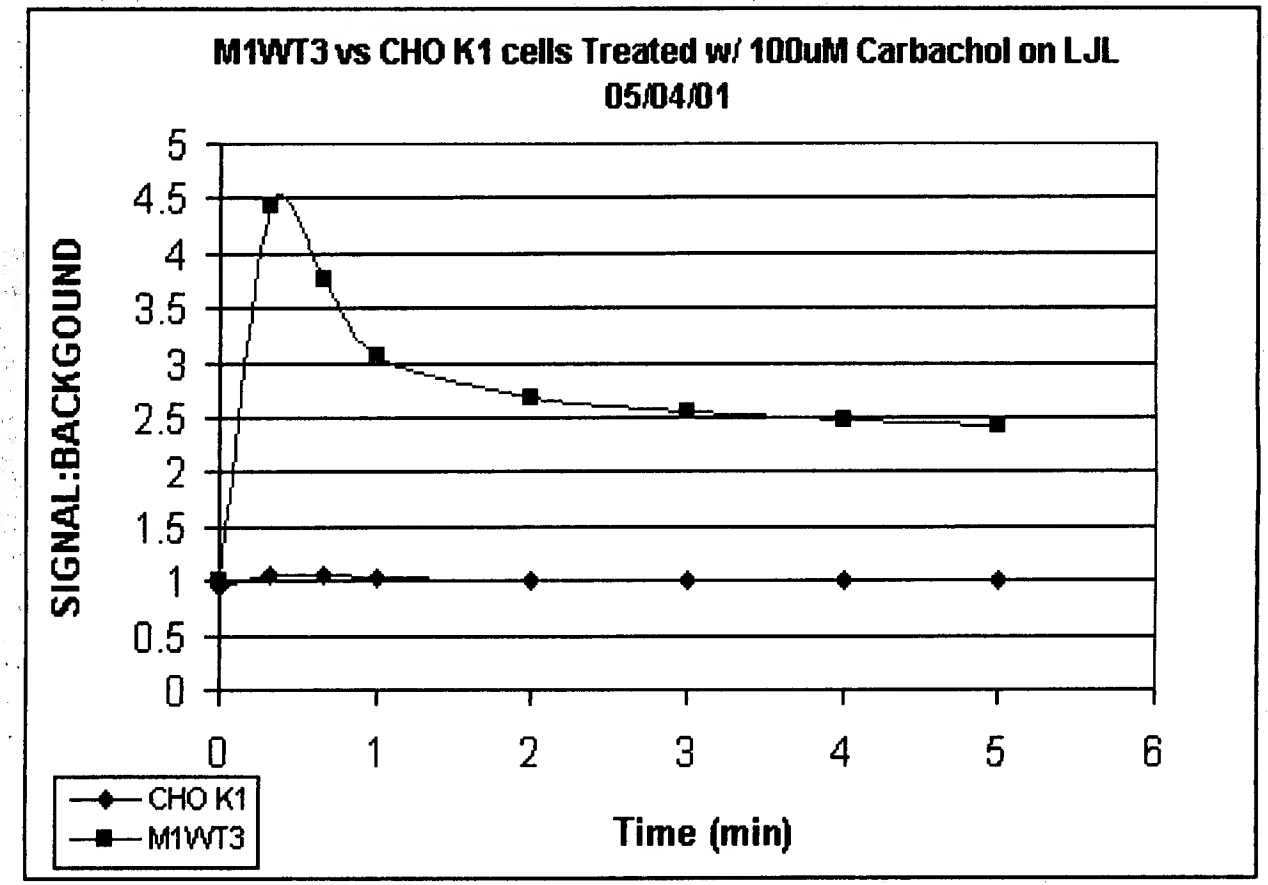
Ca Flux 2º Assay on LJL Analyst



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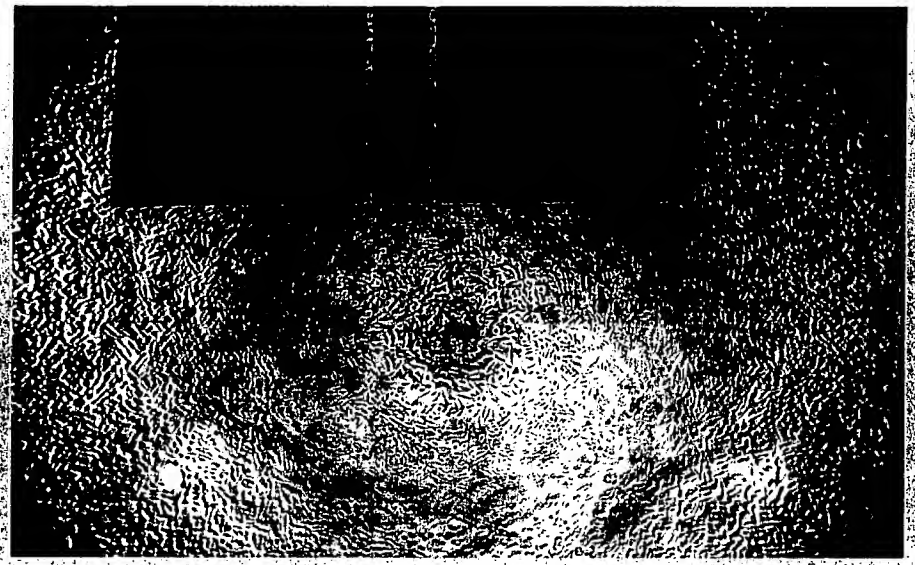
CPW

- 50MHz – 1GHz
- 101 points, -10 dBm
- IF Bandwidth – 10Hz
- SP11 & SP21
- Au & Pt chips
- $5x10^4$ cells/well plated the day before
- Vivian's New Sucrose Buffer

M1 Cells on .505 Pt CPW



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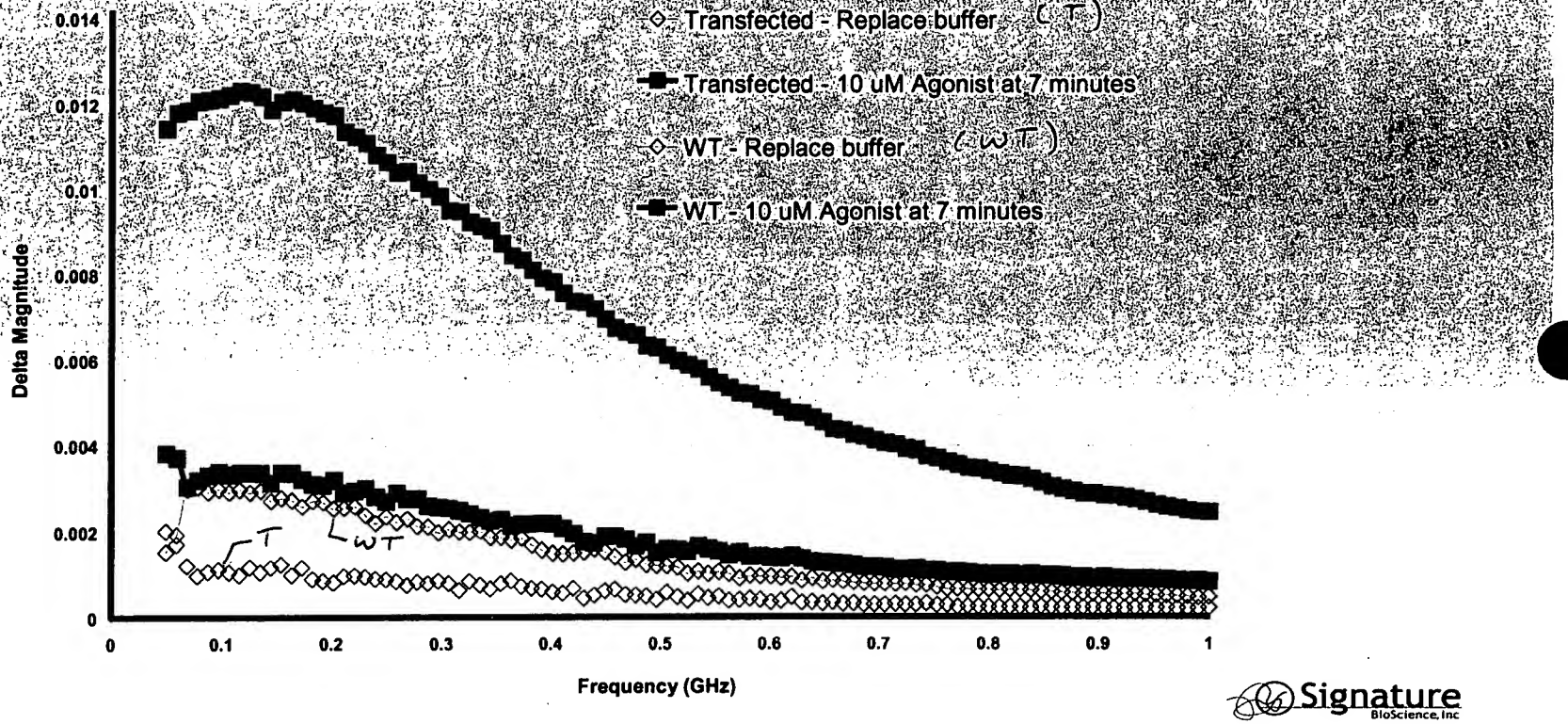
MCS cellular response

- CHO cells – wild type and transfected with well-known GPCR (Gq-coupled)
- Agonist stimulation is seen in transfected cells, not in WT cells
- 2ndary assay: Calcium flux measured in LJL Analyst



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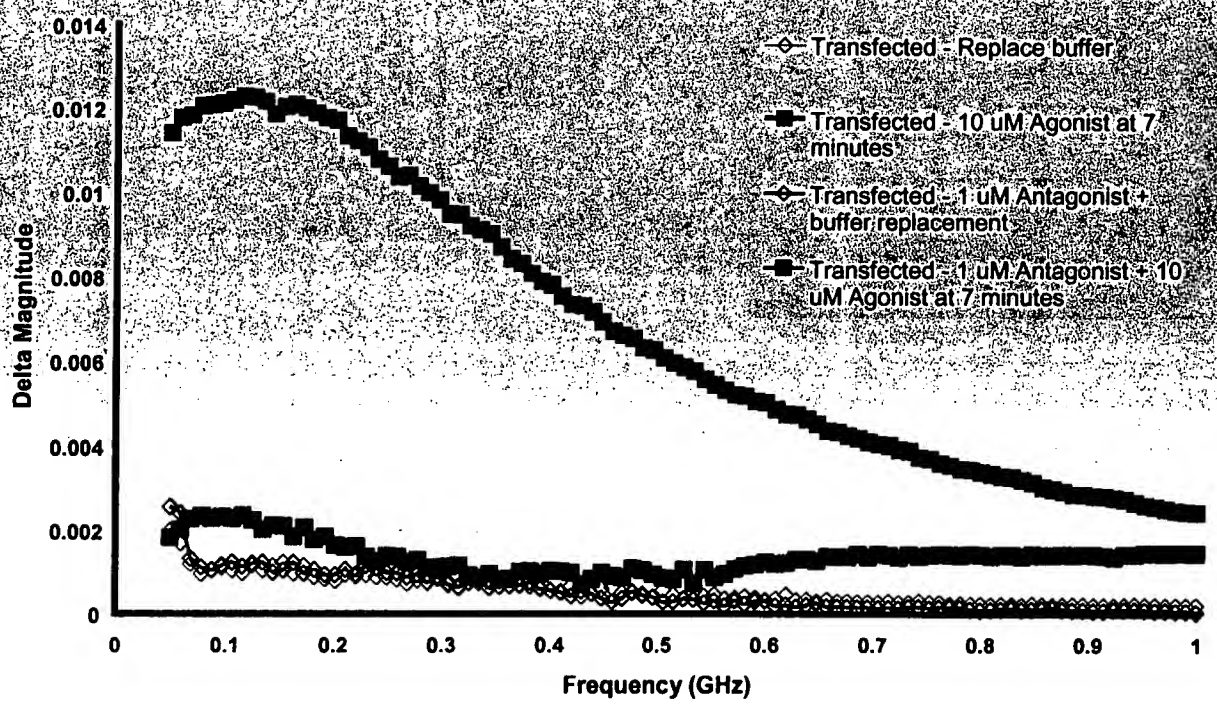
MCS cellular response

- Same cell lines as previous slide
- Agonist stimulation is blocked by pre-treatment with 1 µM antagonist



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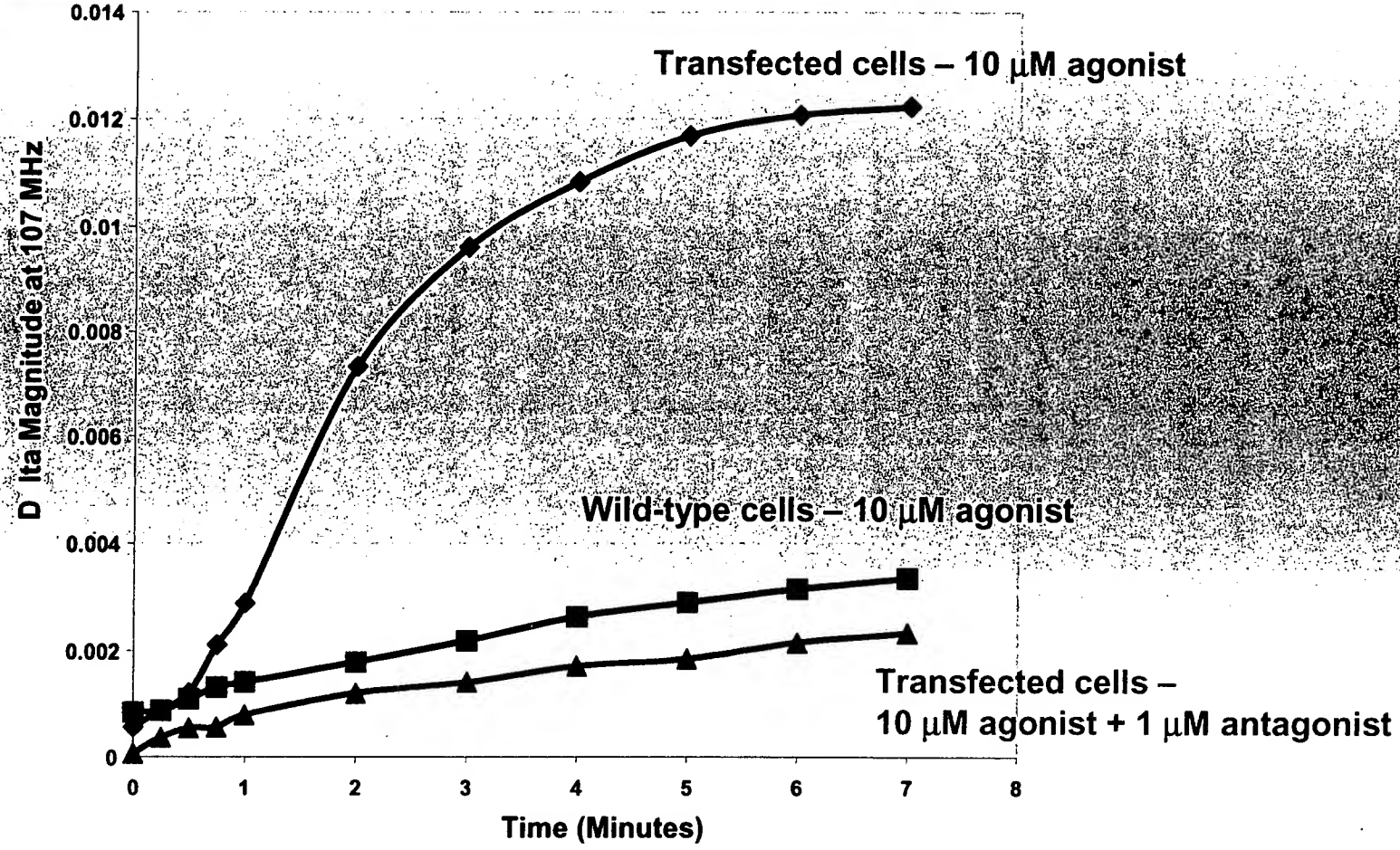
Time course of response to agonist



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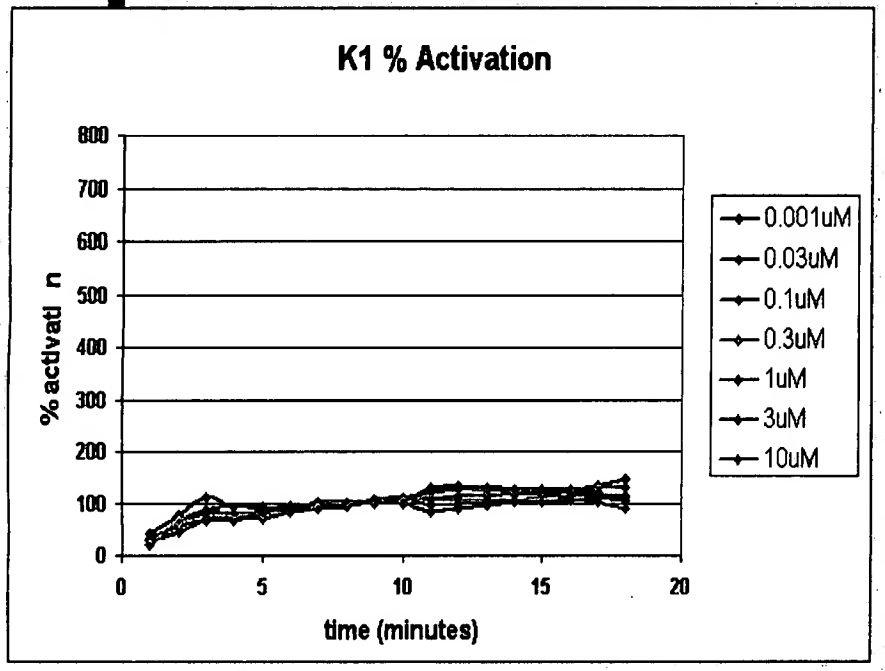
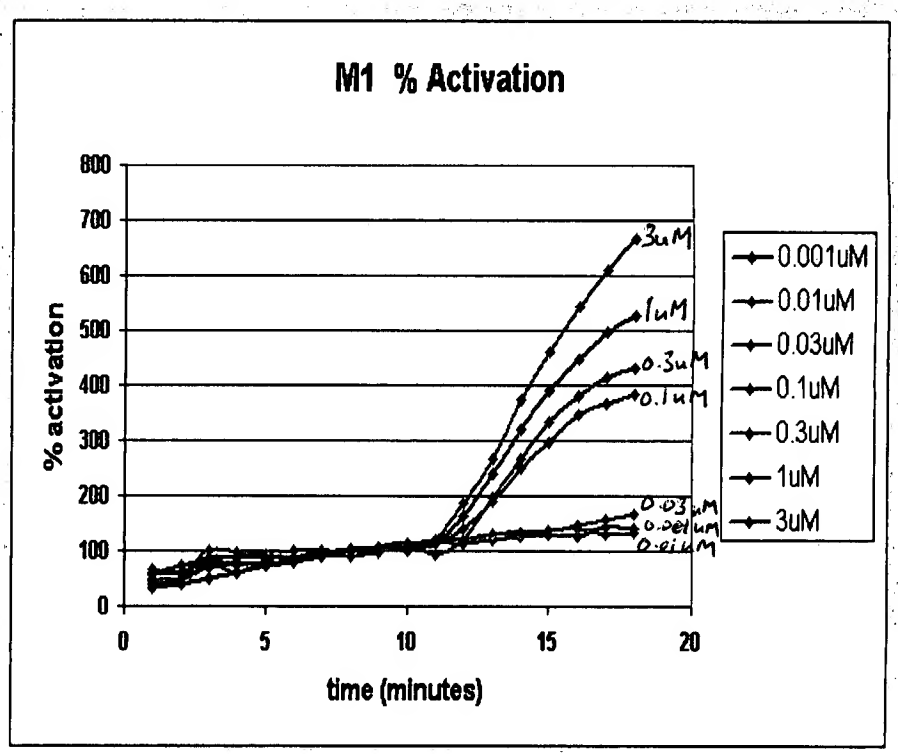
Dose-Response Curves:

CHO-K1 vs. CHO-M1 : carbachol





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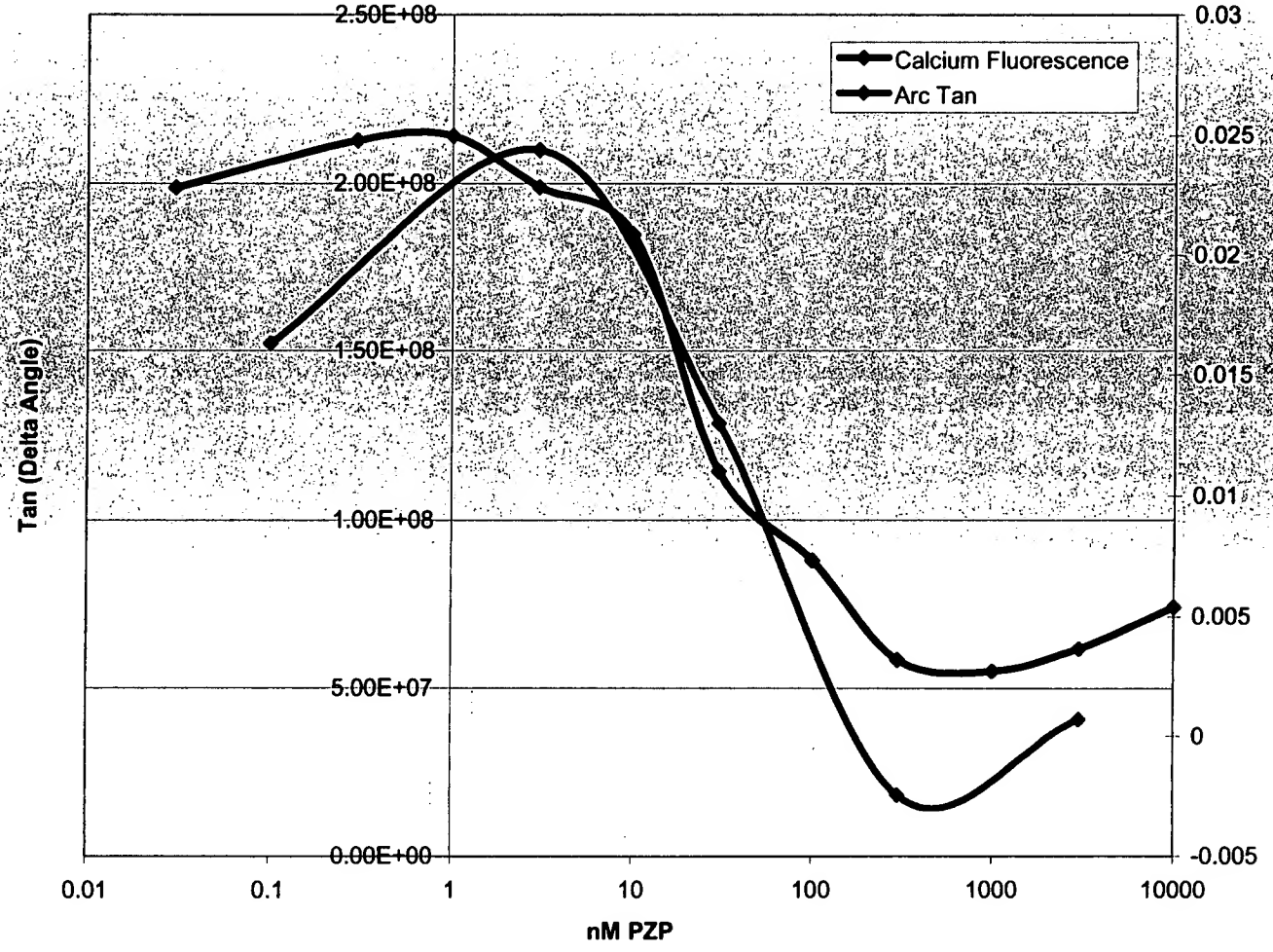
PZP Dose curves … MCS & Ca^{+2} Flux

CHO_{M1} cells treated with 300 nM Carbachol +/- Pirenzepine



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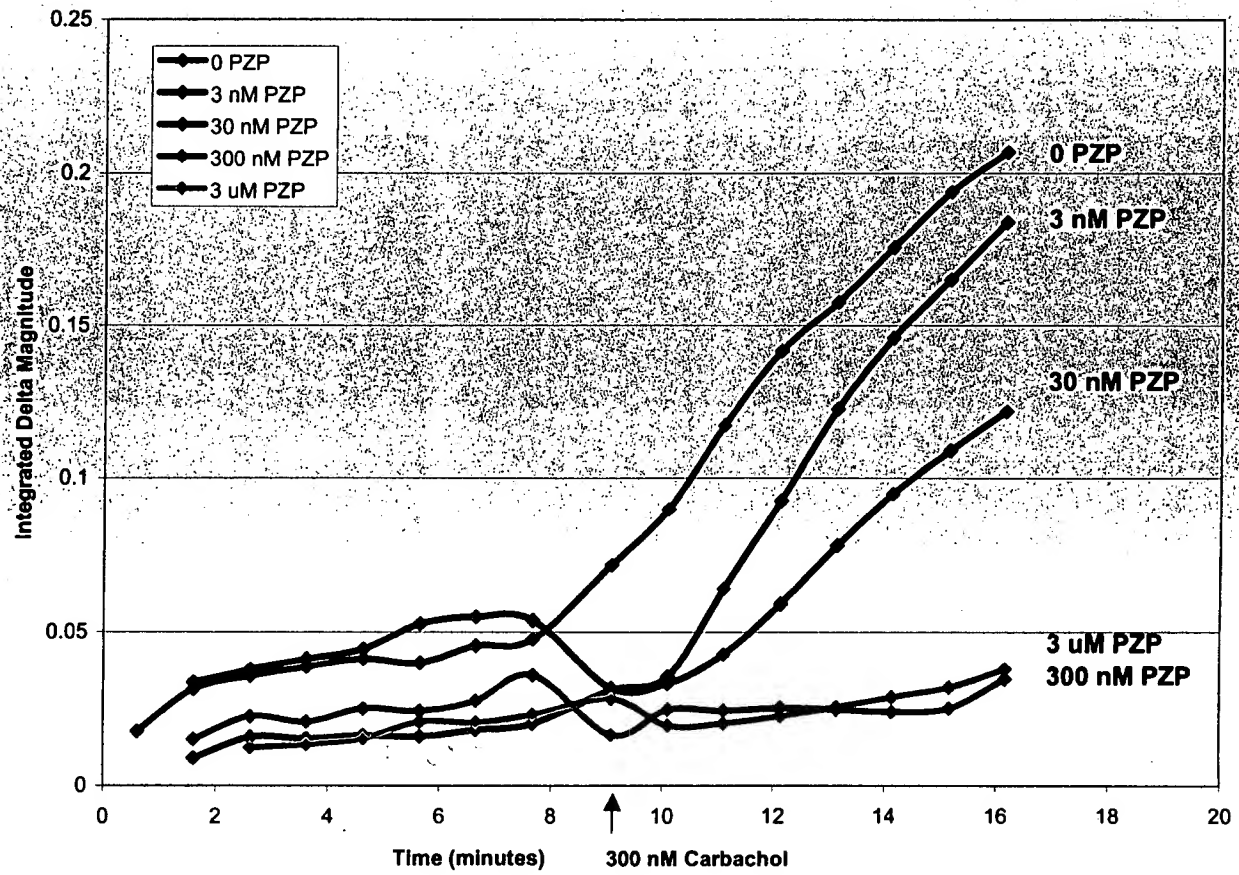
300 nM Carb + PZP

CHO_{M1} cells treated with 300 nM Carbachol +/- Pirenzepine



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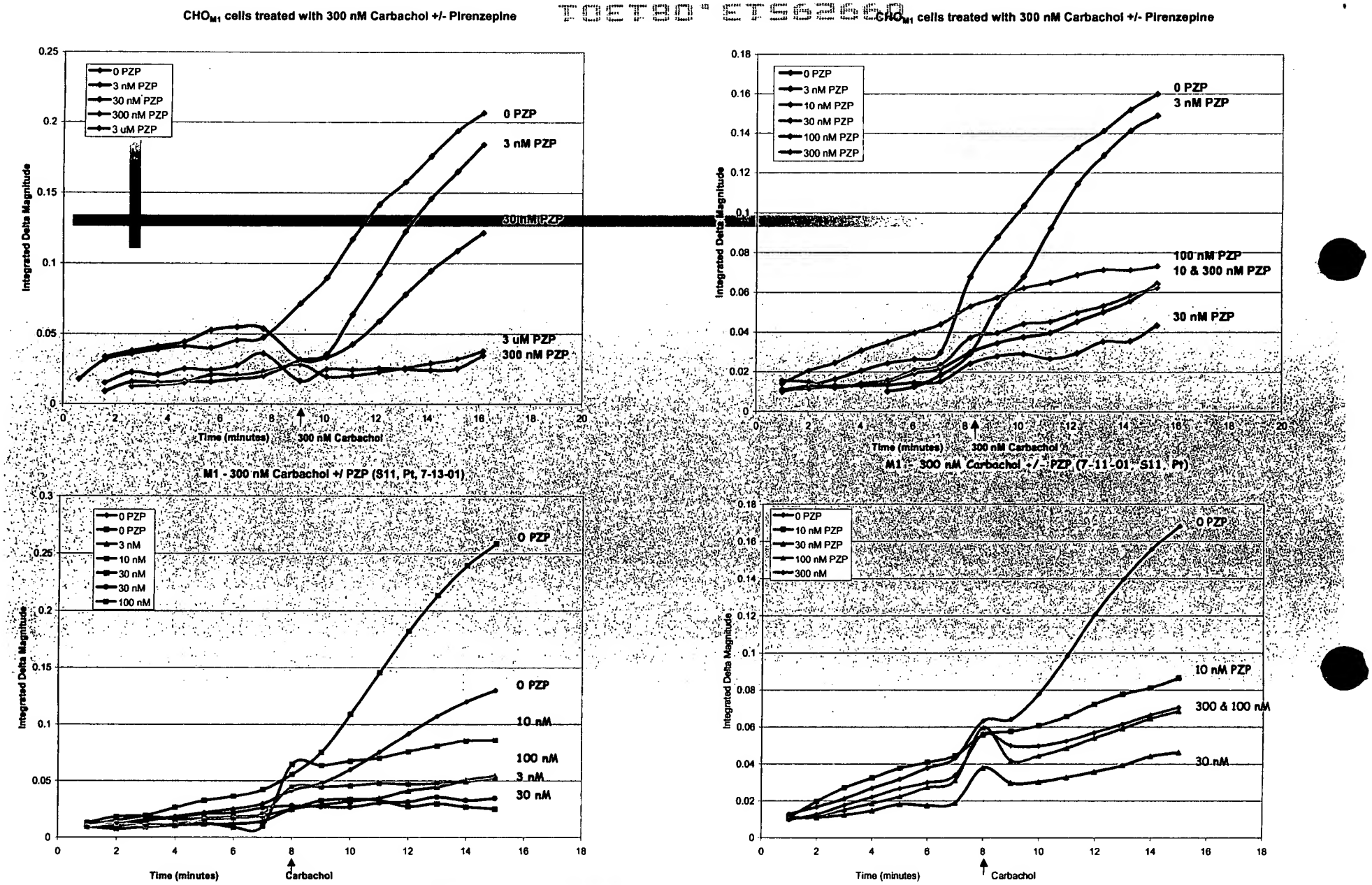
M1 – 300 nM Carb vs PZP Doses

Conclusions:

- PZP always blocks activation by 300 nM Carbachol
- Dose of PZP required to block Carb response varies everyday (look at 3 nM, 10 nM)
- Range of positive response can vary a lot



Roger Plot...

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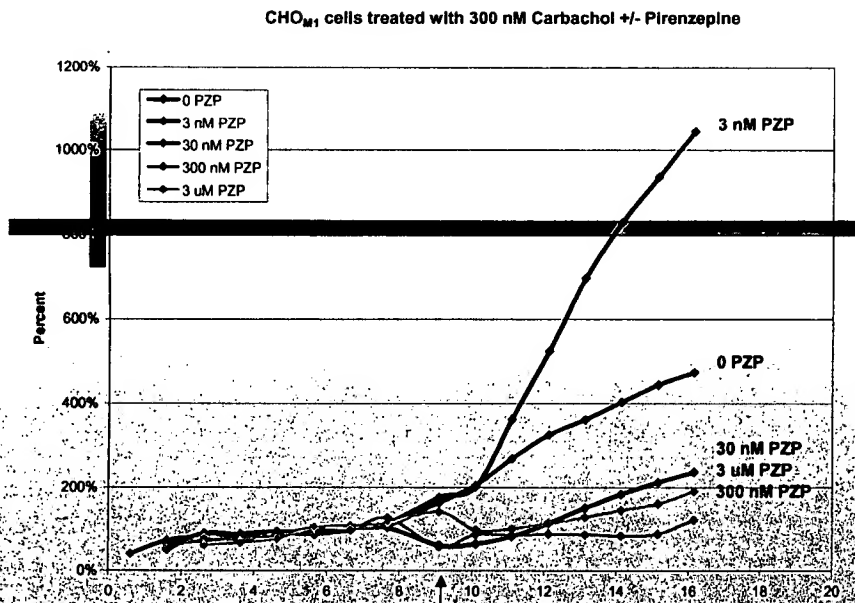

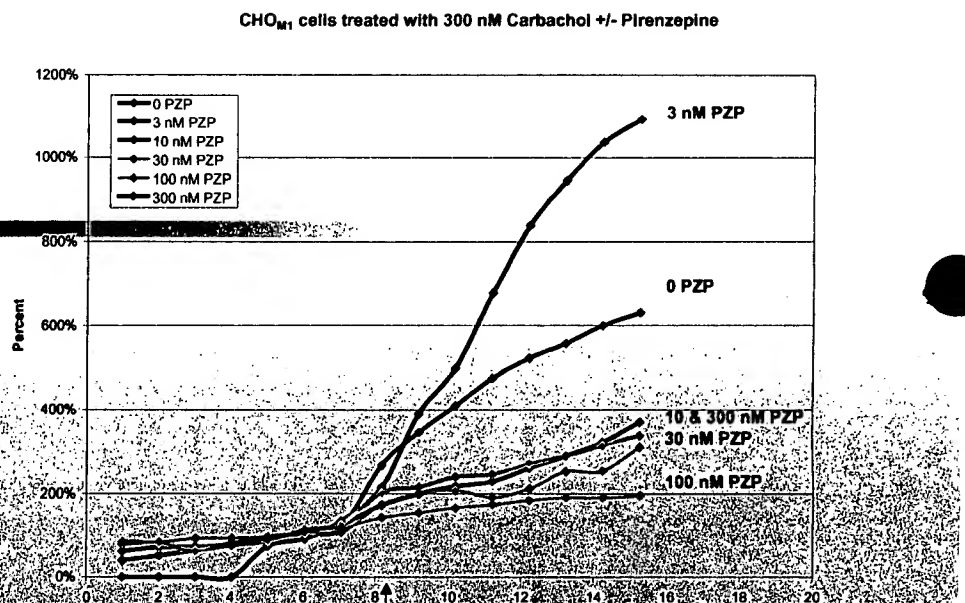

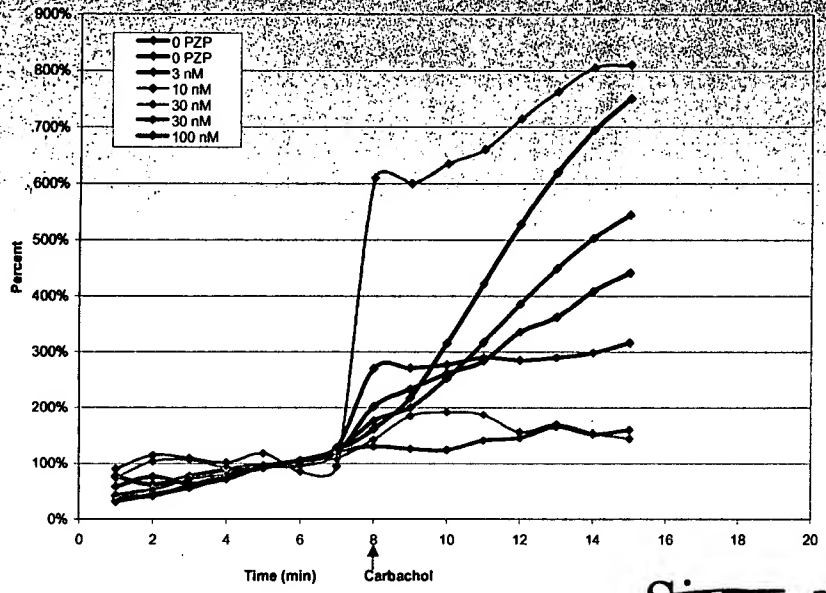

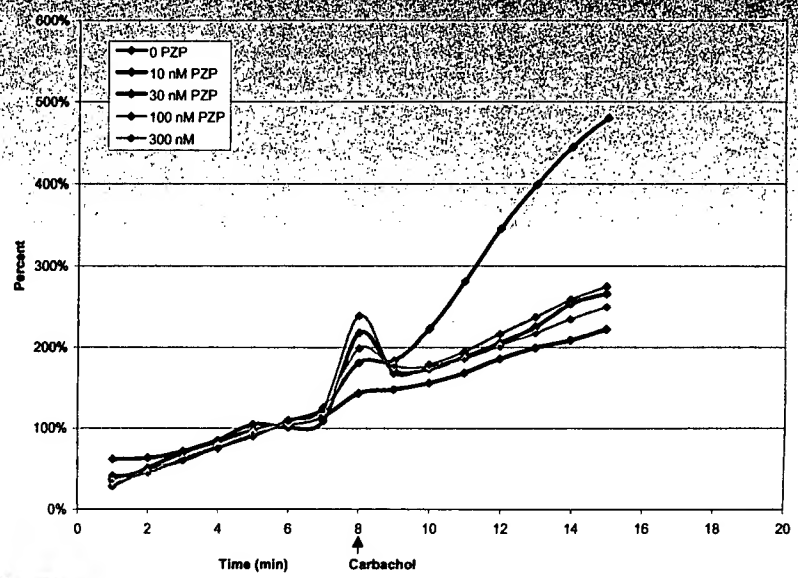

~~Simon plot~~..

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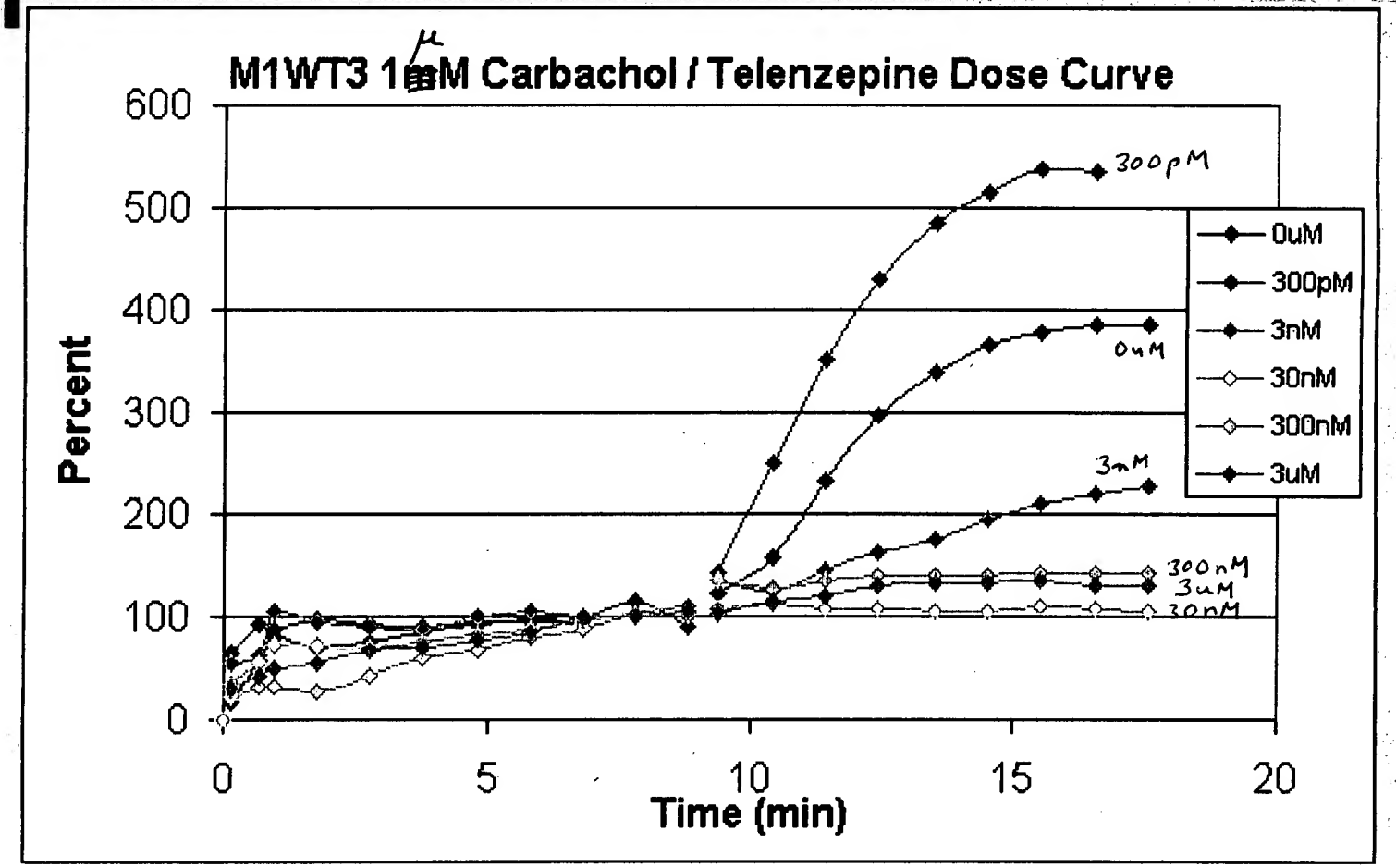
Dose-Response vs. Inhibitor

(Telenzepine)



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